Molecular mechanisms regulating the neuronal architecture in the mouse brain

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von Anita Remus
aus Wolfsburg

1. Referentin oder Referent: Professor Dr. Martin Korte
2. Referentin oder Referent: Professor Dr. Reinhard Köster
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Tagungsbeiträge

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Anita Dreznjak*, Marta Zagrebelsky, Stefanie Rauskolb, Sabrina Schild, Martin Polack, Yves-Alain Barde, Martin Korte: BDNF modulates the postnatal extension of dendrites in the CNS in an area-specific way. 7th FENS Forum of European Neuroscience 2010

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* Anita Remus, geb. Dreznjak

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Abstract

The functional diversity of the brain relies on a very complex neuronal network. The development and maintenance of the neuronal circuitry are tightly regulated through extracellular signals and the translation of these signals into functional and structural changes. In this context, BDNF is known to promote neuronal survival, differentiation and activity-dependent plasticity. While the role of BDNF in regulating the architecture of certain subtypes of peripheral neurons during postnatal development is well documented, the cellular and molecular mechanisms mediating BDNF function in the CNS remained so far mostly unclear. Interestingly, I could show both *in vivo* and *in vitro* that BDNF exerts a cell type-specific effect in regulating neuronal dendritic architecture. Indeed, while inhibitory neurons depend on BDNF for their dendritic development, the dendritic architecture of excitatory neurons is not affected by BDNF depletion. Moreover, this study is especially aimed at understanding the molecular mechanisms underlying the specific effects of BDNF on excitatory *versus* inhibitory neurons throughout the CNS. Notably, I could show that besides BDNF, Zinc is involved in regulating neuronal morphology of excitatory neurons but not of inhibitory neurons through the transactivation of the TrkB receptor in a neurotrophin-independent manner. In the second part of my work, I addressed the molecular mechanisms translating extracellular signals into structural changes at neurons. Therefore, I analyzed the role of the actin binding protein, Profilin2a (PFN2a). PFN2a is known to play a crucial role in modulating actin stability as well as dynamics and is expressed in the central nervous system. However, its role in modulating neuronal architecture is still largely unknown. RNAi mediated knockdown revealed distinct functions for PFN2a in modulating the structure of dendrites and spines, respectively. I could show that PFN2a is involved in maintaining the dendritic structure, and in regulating dendritic spine formation. Moreover, the precise levels of PFN2a are crucial for the spine dynamics and motility. Finally, in this study I could identify PFN2a as a key modulator of activity-dependent structural plasticity at synapses of mature hippocampal neurons.

In summary, I could provide new insights in the molecular mechanism modulating neuronal structure on both from the extracellular and the intracellular side. Zinc as an external messenger is a new molecule to influence neuronal morphology. In addition, the actin binding protein PFN2a is crucial for modulating neuronal structure and plays a key role in activity-dependent structural plasticity.

Zusammenfassung

Die präzisen und diversen Funktionen des Gehirns beruhen auf einem fein abgestimmten Netzwerk von vielen auf vielfältige Art miteinander verbundener Nervenzellen. Die Entwicklung und Aufrechterhaltung dieses komplexen neuronalen Netzwerkes wird durch intrinsische Programme ebenso wie durch extrazelluläre Signale und deren Translation in Veränderungen der neuronalen Morphologie und Funktion reguliert. In diesem Zusammenhang spielt das Neurotophin BDNF eine bedeutende Rolle. Es ist als positiver Regulator für die Morphologie von Nervenzellen sowohl während der Entwicklung als auch im adulten Nervensystem propagiert worden. In dieser Arbeit wurde speziell der zelluläre und molekulare Mechanismus der Funktion von BDNF untersucht. *In vivo* und *in vitro* Untersuchungen der Morphologie von exzitatorischen und inhibitorischen Neuronen zeigten interessanterweise einen so nicht vorhergesagten Zell-spezifischen Effekt von BDNF hinsichtlich der strukturellen Regulation von Neuronen. Der Verlust von BDNF führte zu einer Beeinträchtigung der Dendritenentwicklung in inhibitorischen Neuronen, während die dendritische Komplexität von exzitatorischen Neuronen nicht beeinflusst wurde. Dies konnte auf einen Neurotophin unabhängigen Prozess zurückgeführt werden. Bemerkenswert ist, dass neben der positiv regulierenden Funktion von BDNF Zink ebenfalls an der Aufrechterhaltung der Morphologie von exzitatorischen aber nicht von inhibitorischen Neuronen durch die Transaktivierung des TrkB Rezeptors beteiligt ist. Im zweiten Teil der Studie wurden die molekularen Mechanismen der Übermittlung extrazellulärer Signale in strukturelle Veränderungen von Nervenzellen in den Vordergrund gestellt. In diesem Zusammenhang wurde die Rolle des Aktin-bindenden Proteins Profilin2a (PFN2a) untersucht. Durch eine RNAi-induzierte Hemmung der PFN2a Genexpression konnte eine essentielle Funktion von PFN2a für die Stabilität der Dendriten und für das Wachstum der *spines* nachgewiesen werden. Zusätzlich wurde gezeigt, dass die richtigen Mengen von PFN2a für die Stabilität und Dynamik der *spines* nötig sind. Darüber hinaus konnte ich zeigen, dass PFN2a eine wichtige Rolle in der aktivitätsabhängigen strukturellen Plastizität zugeordnet werden kann. Insgesamt wurden in dieser Arbeit neue Erkenntnisse sowohl in der extrazellulären als auch in der intrazellulären Regulation neuronaler Strukturen gewonnen. Zink konnte als neuer Modulator extrazellulärer Signale identifiziert werden. Desweiteren zeigt diese Arbeit, dass das Aktin-bindende Protein PFN2a ein wichtiger Modulator neuronaler Struktur ist und eine Schlüsselrolle in der aktivitätsabhängigen strukturellen Plastizität einnimmt.

1. Introduction

A hallmark of vertebrate evolution is the development of a complex nervous system composed of over 100 billion neurons in humans. The individual nerve cells are interconnected thereby building a neuronal circuit that allows the control of many different functions, such as speech, movement, emotion or the formation of memory. The brain areas associated with these advanced cognitive functions are the forebrain structures, including the cerebral cortex, the hippocampus, the amygdala and the striatum. Pyramidal neurons are the principal neurons found in most of these mammalian forebrain areas. Moreover, the fact that pyramidal neurons are abundant in all mammals as well as in birds, fish and reptiles indicates preserved core functions und makes understanding the structure and function of these cells crucial to reveal the neuronal basis of sophisticated brain functions (reviewed in Spruston, 2008).

1.1. Pyramidal Neurons

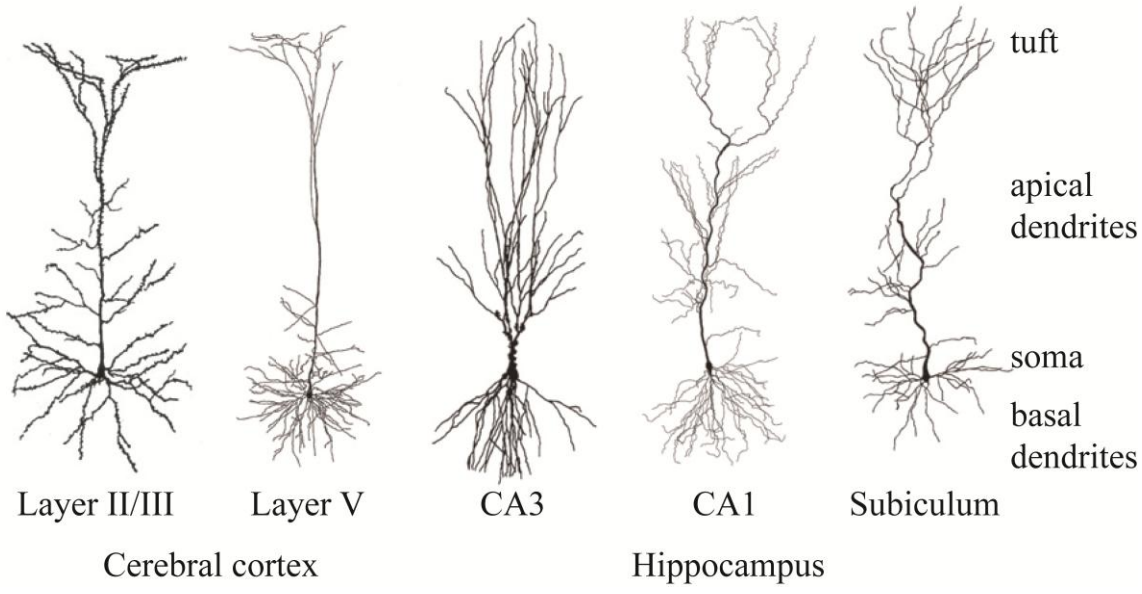

Figure 1 Structure of pyramidal neurons
Each type of pyramidal neuron has characteristic basal and apical dendrites and an apical tuft, however, there are differences between brain areas. The apical dendrite of pyramidal neurons in the cortical layer V is longer but less branched than the apical dendrite of layer II/III pyramidal cells. In hippocampal pyramidal cells, the apical dendrite of CA3 pyramidal neurons branches closer to the soma than that of CA1 pyramidal neurons. All cells displayed are from rat, except the layer III neuron, which is from rabbit (adapted from Spruston, 2008).

All pyramidal neurons in different brain areas are characterized by the pyramidal shaped soma and their distinct apical and basal dendrites (Figure 1) and one axon. While the single apical dendrite is long and connects the soma to a tuft of dendritic branches, the basal dendritic compartment is formed by several relatively short dendrites. Both, apical and basal dendrites are decorated by tiny, protoplasmatic protrusions, the dendritic spines. Pyramidal neurons receive synaptic input at the soma, the axon and dendrites. While the inhibitory GABAergic input is formed at the soma and axon, the dendritic spines receive 90% of all excitatory input. Detailed anatomical studies on fixed brain tissue reveal the extreme variability in dendritic spine shape and size. Dendritic spines can be classified by their shape into three categories: thin, stubby and mushroom-shaped spine types (Figure 2) (Harris et al., 1992). Dendritic spines carry usually one single synapse and are supposed to act as a local synapse specific compartment. Indeed, spines provide a microcompartment for the segregation of postsynaptic chemical responses, such as transient elevations in the intracellular calcium concentration. The prototypical excitatory synapse is defined by the presence of a presynaptic active zone with synaptic vesicles, a well-defined synaptic cleft and a postsynaptic density (PSD) (Harris et al., 1992). Interestingly, it has been found that larger spines accommodate larger postsynaptic densities including more postsynaptic receptors and various organelles, like the spine apparatus, an organelle containing stacks of smooth endoplasmatic reticulum (SER) (Spacek and Harris, 1997). Although the basic features of pyramidal neurons reveal a stereotypical cellular anatomy, they exhibit remarkable fine scale variability among different cortical regions and species. In the cerebral cortex all 6 layers have characteristic pyramidal neurons. The connections between the neurons are highly specific according to both the location of the presynaptic cells and their axons, the location of the postsynaptic cells and their dendrites, and even the location of the synapses onto specific dendritic compartments (Figure 3). The cortical excitatory feedforward pathway shows selective connections between pairs of layer 4

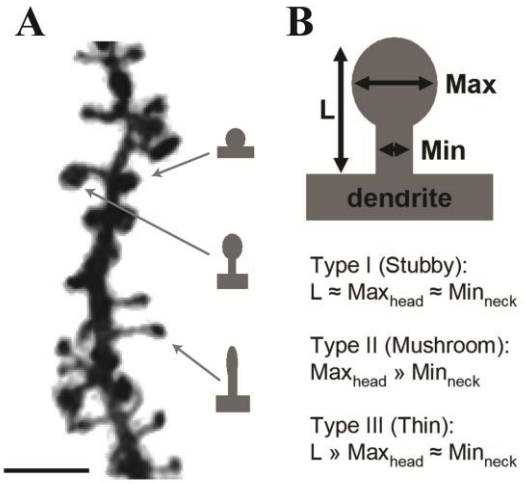

Figure 2 Classification of dendritic spine types
A: Representative image of a dendritic stretch of a hippocampal pyramidal neuron showing the different dendritic spines types. Scale bar, 5µm. **B:** Spine types were classified in three categories, based on spine length (L), the ratio between their maximum head (Maxhead) and minimum neck (Minneck) diameter: stubby (type I), mushroom (type II), and thin (type III) (adapted from Zagrebelsky et al., 2005).

pyramidal neurons, connections from layer 3 to 5, connections within layer 5 and preferential innervations of specific subclasses in layer 6 (reviewed in Bannister, 2005). Interestingly, the excitatory synaptic inputs have different presynaptic origins depending on whether they are located at either distal apical dendrites or proximal apical and basal dendrites. For example, the basal and proximal apical dendrites of layer II/III cells receive inputs from layer IV cells and also receive local-circuit excitation, while the apical tuft of layer II/III cells receives inputs from other cortical areas as well as nonspecific thalamic inputs (Spruston, 2008). The pyramidal neurons in the hippocampus, a region important for learning and memory, are organized in one single layer divided into different subfields (Figure 4). The hippocampal unidirectional network receives its main input to the dentate gyrus *via* the perforant path from the entorhinal cortex. The granule cells of the dentate gyrus transmit the incoming stimuli to the pyramidal cells in the *cornu ammonis (CA)* CA3 area *via* mossy fibers. The CA3 pyramidal cells project in turn *via* the Schaffer collaterals to the CA1 pyramidal cells, from where the output to the entorhinal cortex originate. Interestingly, as described for pyramidal neurons in the cortex, CA1 pyramidal neurons in the hippocampus specifically receive their input from different presynaptic origins. The apical tuft is innervated by the entorhinal cortex, whereas the more proximal dendrites receive input from the Schaffer collaterals from the CA3 region. The basal CA1 pyramidal dendrites get input from CA3 cells close to the CA1 region, whereas CA3 neurons more distant from the CA1 region project primarily to apical dendrites (Ishizuka et al., 1990;Li et al., 1994)

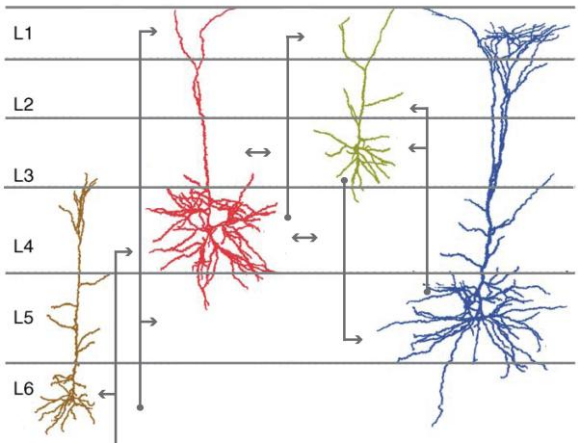

Figure 3 Excitatory feedforward pathways in the cortex
In this highly over-simplified wiring diagram of a cortical column, the cortical information is proposed to enter at layer 4 and layer 6, afterwards entering different cortical layer before activating sub cortical regions or reentering and activating other areas of the cortex (adjusted after Bannister, 2005)

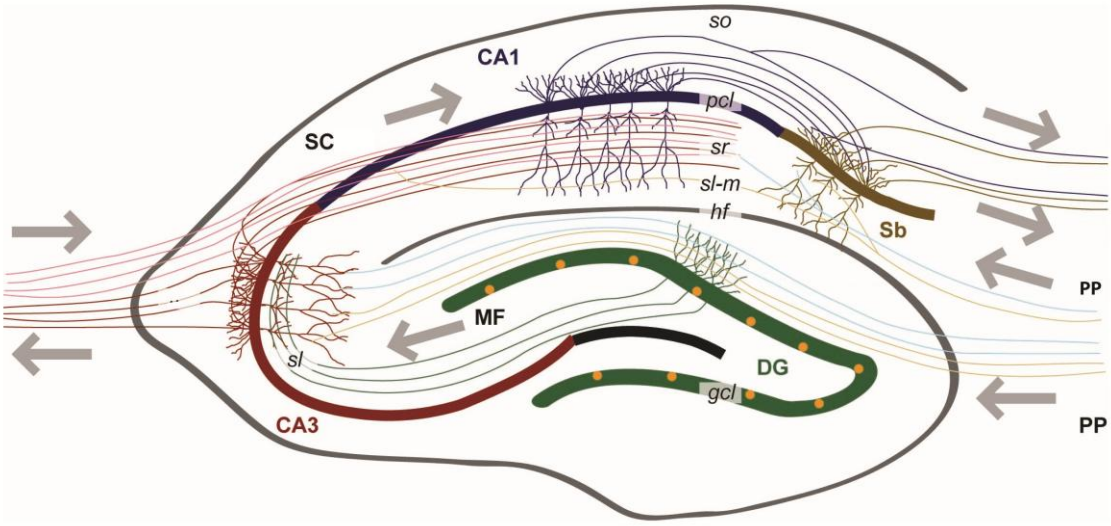

Figure 4 Overview of the hippocampal pathway
The main input arrives from the entorhinal cortex via the perforant path (PP) to the dentate gyrus (DG) granular cells. Their axons, the mossy fibers (MF), project to the CA3 region. From CA3, axons project to CA1 via the Schaffer collaterals (SC). In addition, the perforant path projects directly to the apical tufts of CA1 neurons sending in turn their axons back to the entorhinal cortex (adapted from Neuser, 2010).

1.2. GABAergic neurons

Critical to the function of each pyramidal neuron is the way it responds to synaptic inputs to produce an action potential activating its postsynaptic targets. Thereby the balance of inhibitory and excitatory synaptic input onto pyramidal neurons is crucial. The inhibitory input is provided by interneurons releasing the inhibitory neurotransmitter GABA (γ-aminobutyric acid). In the cortex, interneurons comprise 20% of all neuronal cells, while the hippocampus contains only about 5% interneurons. The main three subtypes of GABAergic interneurons in the cortex and hippocampus can be distinguished by their specific expression of calcium binding proteins: parvalbumin, calretinin or calbindin (Figure 5). GABAergic parvalbumin expressing cells are aspiny inhibitory cells electronically coupled through gap junctions and exert a powerful monosynaptic inhibition onto the principal neurons through multiple perisomatic synapses (McBain and Fisahn, 2001). The calretinin and calbindin expressing cells are medium sized and with few, aspiny, infrequently branching dendrites (Figure 5). Inhibitory neurons receive both excitatory and inhibitory synaptic input as well. Although the total number of synapses (excitatory and inhibitory) is higher on parvalbumin than on calbindin or calretinin expressing interneurons, the ratio of GABAergic inputs is higher on calbindin and

calretinin interneurons when compared to parvalbumin interneurons (McBain and Fisahn, 2001).

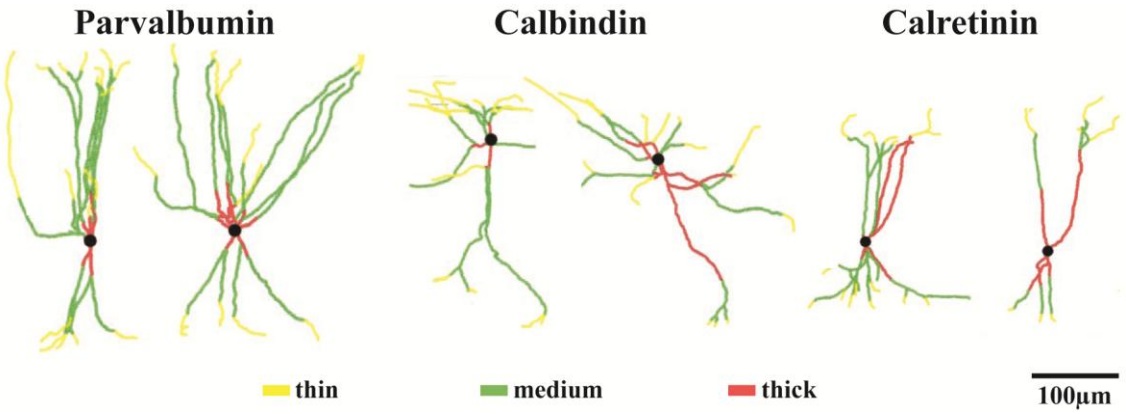

Figure 5 Reconstructed dendritic trees of parvalbumin-, calbindin- and calretinin-containing interneurons
Two examples are shown from each reconstructed cell population, illustrating the characteristics of branching patterns. Different types of dendritic segments separated on the basis of their diameter are indicated with different colors. Parvalbumin cells have the largest dendritic tree, and calretinin cells have the smallest. These cells are reconstructed from interneurons from the CA1 region of the rat hippocampus. Scale bar, 100µm (adapted from Gulyas et al., 1999).

The inhibitory neurotransmitter GABA is not restricted solely to interneurons controlling local circuit properties as in the cortex and hippocampus, but also projecting neurons like the medium spiny neurons (MSNs) of the striatum are GABAergic neurons. The striatum receives input from the entire cortex. The corticostriatal axons mainly innervate the MSNs, which account for the large majority of striatal neurons. The MSNs, in turn, project preferentially to the output nuclei of the basal ganglia or to the external segment of the *globus pallidus* (Figure 6). The activity of MSNs is not solely dependent on cortical excitatory input but also on GABAergic inhibition from the local axon collaterals of MSNs and striatal interneurons (reviewed in Tepper and Bolam, 2004).

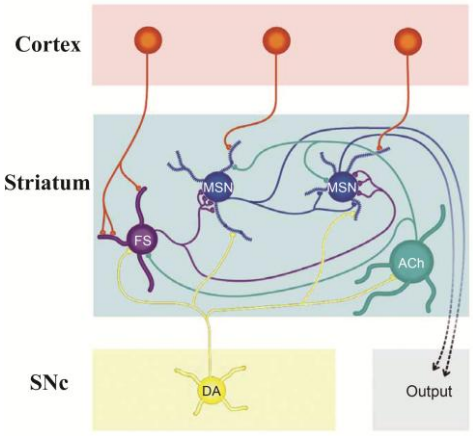

Figure 6 The microcircuit of the striatum
The MSNs, GABAergic and cholinergic interneurons (ACh) receive the most excitatory afferent activity from the cortex. Dopamine neurons (DA) project from the substantia nigra pars compacta (SNc) to the striatum (adujsted after Tepper and Bolam, 2004).

1.3. Structural plasticity

Neural circuits in different brain areas are defined by the architecture of inhibitory and excitatory neurons, including their axons and dendrites ensuring synaptic contact formation and maintenance and therefore a proper neuronal function. During the nervous system development, axons and dendrites are formed in a way which are characteristic for each cell type, thereby establishing the highly specific connections between neurons: the synapses (Katz and Shatz, 1996). While the cellular and molecular mechanisms of axon growth and guidance have been studied intensively (Huber et al., 2003), less is known about dendrite growth (rewieved in McAllister, 2000). Advanced imaging techniques disprove the view of dendrites as intrinsically determined and passive elements during the formation and fine tuning of neuronal networks. Instead, dendritic growth is highly dynamic and can be locally regulated by synaptic activity (reviewed in McAllister, 2000). Changes in dendritic organization and synaptic contacts including spine alteration in shape and number are not only restricted to the development of neuronal circuits. Indeed, learning processes and memory formation during the development and in the adult are crucial for the rapid and coordinated responses to alterations in the environment. Changes in neuronal structure or function, known as synaptic plasticity, are indispensible to modify neural circuits in response to activity generated experience (Lamprecht and LeDoux, 2004). Accordingly, it could be shown that activity modulates the formation and maintenance of dendritic branches in the mature brain (Katz et al., 1989). Rats exposed to an enriched environment exhibited a significant increase in dendritic branching in cortical pyramidal neurons (Volkmar and Greenough, 1972). Furthermore, the dendritic structure can be regulated by the level and integrity of synaptic input. Specifically, elimination of synaptic inputs to a specific set of dendrites caused reduced dendrite branching (Sorensen and Rubel, 2006). Like dendrites, also spines can undergo changes in shape and number in the mature brain, reflecting a change in synaptic connectivity. Especially activity-dependent learning processes, like experience in the enriched environment increased the spine density in the cortex of adult rats (Jones et al., 1997). Structural changes at neurons seem to correlate to functional changes at the synapse. One approach to study this correlation is the strengthening the synapses with induction of long-term potentiation (LTP) or weakening the synapses with the long term depression (LTD). Engert and Bonhoeffer (1999) showed that the induction of LTP in the *stratum radiatum* of CA1 pyramidal neurons in hippocampal slices led to the appearance of new spines in the stimulated region. Spine formation occurred only if persistent, but not transient, changes

in synaptic strength were induced (Engert and Bonhoeffer, 1999). Furthermore, the onset of LTP and the stimulation by glutamate uncaging can be linked to spine enlargement as observed in rat hippocampal slices (Tanaka et al., 2008;Matsuzaki et al., 2004). Interestingly, long-term depression in the same hippocampal region led to shrinkage and even disappearance of spines (Nagerl et al., 2004;Zhou et al., 2004).

Taken together, neuronal activity can influence dendritic structure in the developing and adult brain. However, the molecular signals that translate activity into structural and functional changes remain largely unknown. In that context, neurotrophins have emerged as attractive candidates not only for regulating neuronal differentiation in the developing brain, but also for mediating activity-dependent functional and structural plasticity.

1.4. Neurotrophins and their receptors

Neurotophins were first discovered as secreted factors acting as survival signals between target tissue and the innervating neurons to ensure the matching in size of the target organ and the number of innervating neurons (Purves et al., 1988). Essential for this concept known as the neurotophic theory was the identification and characterization of the Nerve growth factor (NGF) as the first member of the neurotrophin family (Levi-Montalcini, 1987). Brain-derived neurotrophic factor (BDNF) was purified as the second member (Barde et al., 1982) and 2 further neurotrophins were later described in mammals, neurotrophin-3 (NT3) (Ernfors et al., 1990;Hohn et al., 1990) and neurotrophin 4/5 (NT4/5) (Berkemeier et al., 1991). Increasing recent evidence suggests that neurotrophins are not only survival factors, but are also important regulators of neuronal development, function and plasticity (Lewin and Barde, 1996;McAllister et al., 1999). The physiological responses to neurotrophins are mediated by the activation of two different classes of transmembrane receptor proteins, the tropomyosin-related kinase (Trk) family and the p75 neurotrophin receptor (p75 NTR)

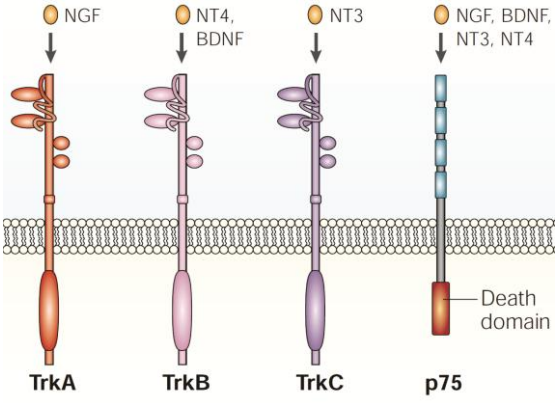

Figure 7 Neurotrophins and their receptors
Neurotrophins bind selectively to specific Trk receptors, whereas all neurotrophins bind to p75. *NGF*, nerve growth factor; *BDNF*, brain derived neurotrophic factor; *NT*, neurotrophin (adapted from Chao, 2003).

(Figure 7) (Reichardt, 2006). The Trk receptor family includes TrkA, TrkB and TrkC. The TrkA receptor can be activated preferentially by NGF, TrkB by BDNF and NT4, while TrkC displays preference for NT3. In contrast to the highly specific binding to the Trk receptors, all neurotrophins bind with equal affinity to the $p75^{NTR}$. Neurotrophins are synthesized as proforms that can be cleaved intracellularly to release mature ligands (Seidah et al., 1996b;Seidah et al., 1996a). Notably, recent data suggest that proneurotrophins can be released as well and bind with higher affinity to the $p75^{NTR}$ promoting apoptosis, while mature neurotrophins preferentially bind to Trk receptor thereby inducing cell survival (Lee et al., 2001). However, so far it is still not clear if endogenous proneurotrophins are indeed released under physiological conditions or not (Matsumoto et al., 2008;Yang et al., 2009;Nagappan et al., 2009).

Hence, the biological function of neurotrophins is regulated by their tissue-specific expression levels as well as their proteolytic cleavage. Moreover, signaling *via* their dual receptor system results in the extremely wide spectrum of neurotrophin actions possibly due to activation of diverse intracellular signaling cascades following ligand binding.

1.4.1. TrkB signaling and transactivation

Tyrosine kinase-mediated signaling by the TrkB receptor promotes survival and differentiation of several neuronal populations. In detail, upon ligand binding (BDNF or NT4), TrkB receptors dimerize and their cytoplasmatic domain becomes catalytically active. Specifically, the vertebrate Trk receptors contain 10 evolutionarily conserved tyrosines in their cytoplasmic domains, three (Y670, Y674 and Y675 in human TrkA sequence) are in the autoregulatory loop of the tyrosine kinase domain, controlling tyrosine kinase activity (Huang and Reichardt, 2003). Phosphorylation of two tyrosines (Y490 and Y785) not localized in the kinase activation domain activate the docking sites for Src homologous protein (Shc) and phospholipase C-γ1 (PLC-γ1), respectively (Stephens et al., 1994). Phosphorylated Y490 recruits and phosphorylates Shc *via* a PTB (phosphotyrosine binding) domain (Dikic et al., 1995) whereupon phosporylated Shc activates the Ras/Raf/MEK/MAPK pathway (Kaplan and Miller, 2000) inducing differentiation and neurite growth. Furthermore, phosphorylated Shc recruits a complex of Grb-2 and the Ras exchange factor SOS which activates the PI3K (phosphoinositide 3-kinase) pathway (Atwal et al., 2000) thereby mediating the survival function of

neurotrophins. Phosphorylation of Y785 recruits PLC γ1 which catalyses the cleavage of the substrate PIP2 to DAG and IP3 thereby promoting activity-dependent plasticity (Figure 8) (Vetter et al., 1991).

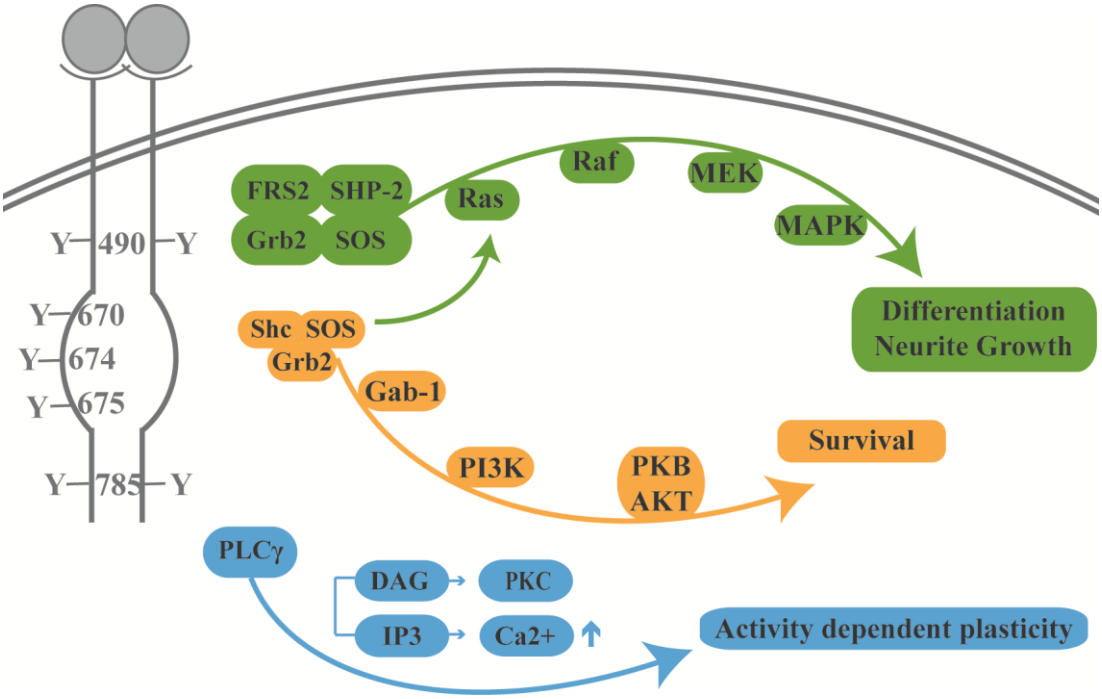

Figure 8 Signaling through the Trk receptor
Neurotrophin binding to Trk receptors triggers dimerization leading to the activation of different signaling pathways through phosphorylation of tyrosin residues (Y490 and Y785) and recruitment of various adapter molecules. Shc/Grb2/SOS and the FRS2/SHP-2/Grb2/SOS complex are two complexes of adapter molecules. Ras/Raf/MEK/MAPK induces the differentiation of neurons and neurite growth, whereas the PI3K/AKT pathway mediates the survival functions. The phosphorylated tyrosine (Y785) in the C terminus recruits PLCγ, which leads to release of calcium from internal stores (adjusted after Bibel and Barde, 2000).

Although ligand-induced dimerization of TrkB receptors is a well-known mechanism for neurotrophin signaling, increasing evidence indicates that biological responses can in addition be mediated without the involvement of neurotrophin ligands. (Carpenter, 1999) The ligand-independent activation of TrkB receptors is called transactivation (Figure 9). The first hint for a neurotrophin-independent TrkB activation was the observation that a conditional deletion of BDNF prevented the enhanced tyrosine phosphorylation of TrkB during epileptogenesis (He et al., 2004), while the NT-4 was not involved in epileptogenesis (He et al., 2006). Subsequent studies performed in cell culture provided evidence that TrkB can be transactivated by G protein-coupled receptor (GPCR) ligands like adenosine (Lee and Chao, 2001). So far, the studies about the transactivation *via* adenosine were focused in application adenosine in neurodegenerative and injury conditions (Wiese et al., 2007). However, while the physiological role of GPCR ligand

transactivation is still unknown, further studies described the transactivation of the TrkB receptor through the Src family kinase (SKF) *via* the divalent cation zinc (Huang et al., 2008;Huang and McNamara, 2010) to occur in physiological conditions. Zinc is stored in, and released from, the presynaptic vesicles of some glutamatergic neurons in the cerebral cortex, including the neocortex, amygdala and is especially concentrated in the mossy fibers of the hippocampus. Efferent zinc-containing fibers from these regions project almost exclusively to (1) cerebral cortex and amygdala (2) striatum and (3) septum, nucleus of the diagonal band and medial hypothalamus (Frederickson et al., 2000).

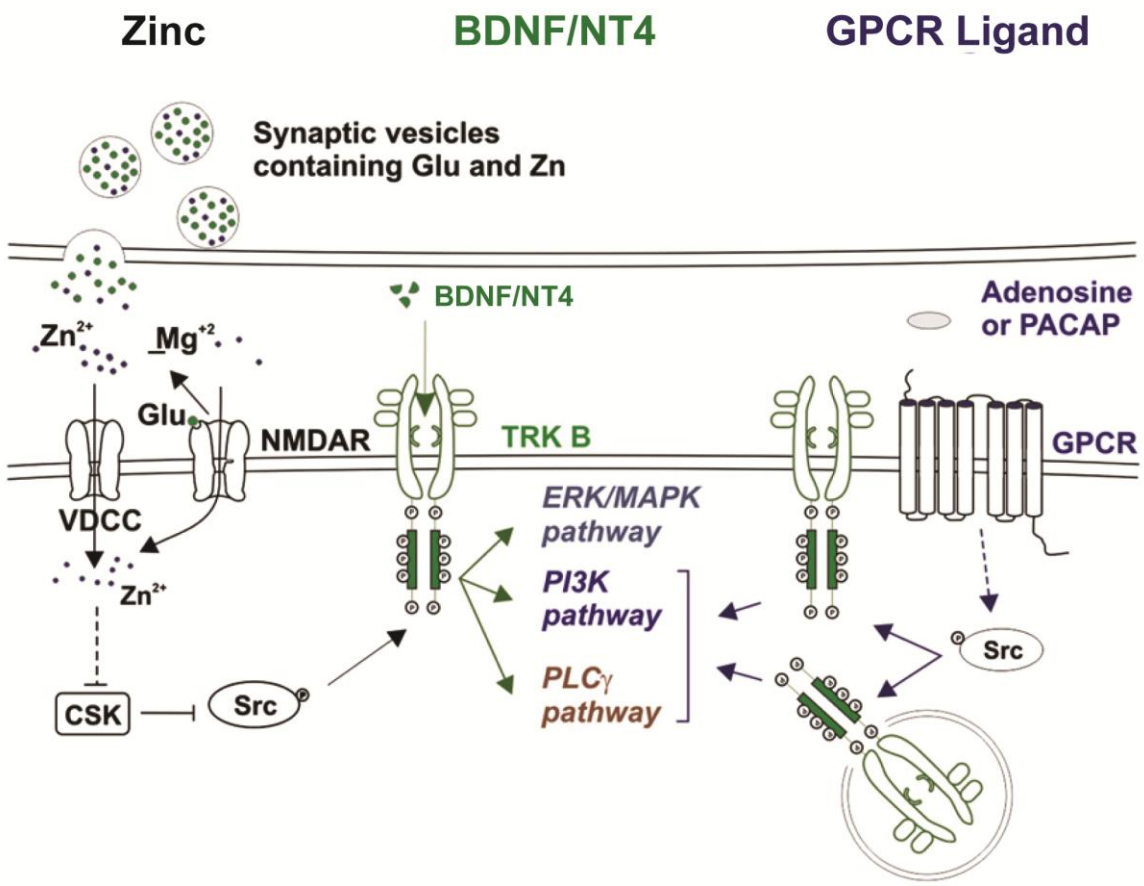

Figure 9 BDNF/NT4-dependent and independent mechanism of TrkB activation
TrkB activation via BDNF or NT4 binding to cell surface TrkB, followed by receptor dimerization, autophosphorylation (minutes), and finally activation of the downstream signaling pathways (ERK/MAPK, PI3K, PLCγ). In contrast, GPCR ligands (adenosine, PACAP) indirectly transactivate Trk receptors (hours) through Src kinase. TrkB can undergo a transactivation via Zinc as well by entering the postsynaptic neuron through NMDAR or VDCC, zinc activates Src kinase to phosphorylate and transactivate TrkB (minutes) by inhibiting CSK kinase (adjusted after Nagappan et al., 2008).

Zinc can be released together with the excitatory transmitter glutamate upon physiological stimulation (Frederickson et al., 2005), thereby entering postsynaptic neurons through voltage-gated calcium channels (VGCCs) and NMDA receptors (Huang et al., 2008). Following its entry, Zinc can activate Src family kinases (SFK) (Src, Fyn

and Yes) by inhibiting their autoinhibition (Huang et al., 2008). Zinc is thereby promoting preferential phosphorylation of, Tyr-705/Tyr-706 and the PLCγ phosphorylation, by a SFK-dependent but TrkB kinase-independent mechanism, a critical signaling event for transactivation of TrkB via Zinc (Huang and McNamara, 2010).

In sum, the TrkB receptor can be activated by two distinct mechanisms, a neurotrophin (BDNF or NT4)-dependent and a neurotrophin-independent mechanism, including the Adenosine or Zinc induced TrkB transactivation described above. However, despite the fact that BDNF and Zinc activate TrkB in a similar manner, the biological relevance of TrkB transactivation by Zinc remains unclear.

1.4.2. BDNF and its role in modulating neuronal morphology

The TrkB receptor ligand BDNF is widely expressed in the CNS. Its expression levels are low during fetal development, markedly increase after birth and then decrease to adult levels (Maisonpierre et al., 1991), with the highest levels found in the hippocampus followed by the cerebral cortex. Moreover, in some CNS structures, such as the striatum and the spinal cord expressing very low levels of BDNF mRNA (Hofer et al., 1990;Kolbeck et al., 1999) the BDNF protein can be detected indicating its anterograde transport from other areas (Altar et al., 1997). Beyond the well described effects on neuronal survival (Barde et al., 1982), BDNF influences almost all aspects of the nervous system development (reviewed in Huang and Reichardt, 2001). In addition to actions on dendritic growth of excitatory pyramidal neurons in the developing visual cortex (McAllister et al., 1995;McAllister et al., 1996;McAllister et al., 1997;Horch et al., 1999), it has been reported that BDNF regulates as well the development of GABAergic neurons. Specifically, BDNF promotes the differentiation of GABAergic neurons in the hippocampus and the striatum (Ip et al., 1993;Mizuno et al., 1994;Marty et al., 1996;Ivkovic and Ehrlich, 1999;Yamada et al., 2002) and regulates dendritic development of hippocampal and cortical GABAergic neurons in culture (Vicario-Abejon et al., 1998;Kohara et al., 2003). Furthermore, BDNF facilitates the formation and maintenance of both excitatory (Rutherford et al., 1998;Vicario-Abejon et al., 1998;Tyler and Pozzo-Miller, 2001) and inhibitory synapses (Seil and Drake-Baumann, 2000;Marty et al., 2000) as well as spine formation (Tyler and Pozzo-Miller, 2003). Moreover, it has been shown that BDNF promotes excitatory and inhibitory synaptic transmission in

culture (Matsumoto et al., 2006). One of the clearest indications that BDNF modulates synaptic transmission comes from the analysis of its role during the induction and maintenance of long-term potentiation (LTP) (Korte et al., 1995;Patterson et al., 1996).

Since increasing evidence suggests an activity-dependent regulation of dendrites and spines (see above), neurotrophins and especially BDNF became an attractive and important candidate in this context to regulate the neuronal shape. Indeed, both its biosynthesis and secretion occur in an activity-dependent manner in neurons (Thoenen, 1995;Poo, 2001). However, so far it was difficult to investigate the role of BDNF during the postnatal development and in the mature central nervous system. Conventional BDNF knockout mice in fact die too early to be used to analyze the role of BDNF after its marked postnatal increase (Maisonpierre et al., 1990; Zafra et al., 1990; Castren et al., 1992). To circumvent this difficulty, a number of mouse lines have been generated using cre-mediated excision of *bdnf* (Rios et al., 2001;Gorski et al., 2003;Baquet et al., 2004;He et al., 2004;Chan et al., 2006;Chan et al., 2008;Monteggia et al., 2007;Unger et al., 2007). For example, in early-onset forebrain-restricted BDNF conditional knockout mice (*Emx*-BDNFko), (Gorski et al., 2003;Baquet et al., 2004) while the cortical and striatal volume are reduced at 4 months of age, the hippocampal volume was not altered. Additionally, a reduced dendritic complexity of cortical excitatory pyramidal neurons of layer II/III and inhibitory medium spiny neurons at 3 weeks could be described in the *Emx*-BDNFko mice. Baquet and colleagues (2004) suggested that BDNF expression is necessary for the maintenance of pyramidal dendritic structure in the cortex as suggested also by the strong increase in BDNF expression in the cortex at 3 weeks of age. Furthermore Baquet (2004) claimed that the impaired morphology of MSNs in these mutants might be due to the loss of cortical BDNF early in development and thereby of its anterograde transport to the striatum, as suggested previously (Altar et al., 1997). However, the interpretation of these results is complicated by the fact that all these BDNF conditional knockout mice were restricted to a specific brain area and did not take into account the anterograde axonal transport of BDNF. Therefore, a new conditional mutant mouse *(cbdnf ko)* lacking BDNF throughout the CNS was generated through the use of a cre recombinase inserted in the *tau* locus, a gene expressed exclusively in postmitotic neurons (Tucker et al., 2001) (Rauskolb et al., 2010) to allow the exploration of the role of BDNF both in the CNS postnatal development and in the maintenance of neuronal morphology. These BDNF-depleted animals survived for several months after birth and while the size of their brain was reduced, the effect of BDNF deprivation was surprisingly area-specific. Indeed in 8

weeks old *cbdnf ko* mice, while the volume of the hippocampus was not significantly affected, the size of the cortex was reduced by 20% and the one of the striatum was even further reduced to 35%. Interestingly, the volume reduction of the striatum could not be explained by cell loss. However, a detailed analysis showed a highly significant reduction in the dendritic length and complexity as well as in the spine density of mature inhibitory medium spiny neurons. On the contrary, the analysis of the morphology of mature excitatory CA1 pyramidal neurons of *cbdnf ko* mice revealed only minimal changes on dendritic length and branching. In addition, while spine density was not changed in the *cbdnf ko* mice, the proportion of mushroom-type spines was significantly decreased (Rauskolb et al., 2010)

Taken together, the TrkB receptor ligand BDNF plays an important role in neuronal survival, growth and maintenance in several neuronal systems (Bibel and Barde, 2000), and participates in plasticity processes important for learning and memory (Korte et al., 1995;Patterson et al., 1996). Interestingly a global deprivation of BDNF in the CNS reveals an unexpected selectivity requirement for BDNF during the normal postnatal development of different brain areas.

However, to understand how neuronal morphology can be adjusted upon activity-dependent influences, besides the neurotrophin and receptor interaction at the cell membrane, the cell cytoskeleton needs to be involved.

1.5. Actin cytoskeleton

The molecular mechanisms mediating the activity-dependent modifications of neuronal structure both in the adult brain and during developmental are poorly understood. Recent findings are beginning to suggest that the cytoskeleton might play a crucial role in mediating the changes in neuronal structure. The cytoskeleton is formed by a heterogeneous network of filamentous structures in neurons, including neurofilaments, microtubules and microfilaments. The latter is the most dynamic cytoskeletal component and consists of actin molecules present in two forms, the monomeric globular G-actin and the polymeric filamentous F-actin. The F-actin filaments generally turn over within 2 min (Star et al., 2002) by a process of continuous “treadmilling”. This steady-state process involves the continuous polymerization of G-actin at the barbed end (growing end) of the filament toward the plasma membrane and depolymerization of F-actin at the opposite,

pointed end (Pollard and Borisy, 2003). Consistent with the observation that actin plays a role in cell motility (Cooper, 1991), actin microfilaments are found in the entire neuron, but are especially concentrated at dynamic structures, like presynaptic terminals, growth cones and dendritic spines. In line with the observation that dendritic spines can undergo rapid changes upon activity (Yuste and Bonhoeffer, 2001), advanced techniques using fluorescently labeled actin showed an activity-dependent turnover of filamentous actin in spines (Star et al., 2002). This indicated a fast rearrangement of actin in dendritic spines and confirming its capacity for driving changes in morphology. Previous reports could shed light onto the underlying mechanisms mediating actin reorganization in dendritic spines upon activity stimulation as well as basal actin dynamics under physiological conditions. It was observed that the actin cytoskeleton organization varied in different spine compartment to allow stable as well as dynamic spine morphology (Figure 10). Specifically, dendritic spine base, neck and head consist of a mixture of branched and linear actin filaments. The spine base and neck contained a network of long and short branching F-actin filaments, while most branched and very dynamic actin filaments were localized in the spine head just underneath the post synaptic density (PSN) (Korobova and Svitkina, 2010). Detailed analysis observed three different F-actin pools in dendritic spines, the dynamic pool, the stable pool and the enlargement F-actin pool (Honkura et al., 2008). The dynamic pool polymerized near the tip of the spines and treadmilled towards the center of the spine. The stable F-actin pool was located at the base of the spine and varied depending on spine volume. The third pool, termed the enlargement pool, was formed during the enlargement of spine upon glutamate uncaging and was distributed throughout the spine head. Moreover, upon activity-dependent stimulation, the ration of monomeric G-actin and filamentous F-actin was altered. The induction of LTP caused an increase in spine volume and shifted the G-actin/F-actin ratio toward F-actin (Fukazawa et al., 2003), whereas LTD resulted in the opposite effect, namely a shrinkage of spines and a shift in toward G-actin (Okamoto et al., 2004). Interestingly, studies inhibiting actin dynamics showed suppressed LTP, suggesting that a

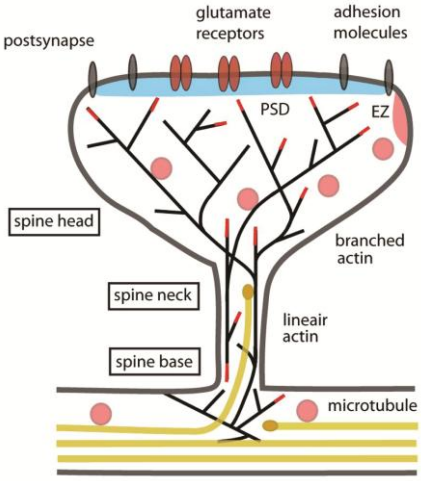

Figure 10 Actin organization in spines
Actin organization is different in the spine head and the spine neck/base. Branched actin is observed in spine head, while linear actin is located in the spine neck/base (adapted from Hotulainen and Hoogenraad, 2010).

dynamic actin cytoskeleton is involved in mediating synaptic plasticity (Krucker et al., 2000). Taken together these studies indicated that spines have an elaborate actin dependent mechanism regulating their formation as well as stability of their size and shape.

A key feature of the actin function is its ability to support both, motile and stable structures. During neuronal development the actin cytoskeleton is involved in activity-dependent process for the correct building of neuronal circuits. Additionally, the fact that actin is highly concentrated in dendritic spines in the adult brain with different F-actin pools, the stable and the dynamic pool, support a stability function and the possibility for morphological plasticity as well. (Matus, 2000;Cingolani and Goda, 2008).

Actin exists in a dynamic balance between two forms, the monomeric globular form (G-actin) and the filamentous form (F-actin). The simple steady state mechanism of assembly and disassembly does not explain the high degree of spatial and temporal regulation of actin dynamics in cells. Thus, the actin dynamic underlie several actin-binding proteins to influence the structure and organization of the actin cytoskeleton.

1.5.1. Profilin

The small protein profilin was among the first actin-binding proteins to be discovered and is supposed to play a key role in the regulation of actin dynamics by acting as a nucleotide exchange factor for G-actin and thereby facilitating the growth of actin filaments (Carlsson et al., 1977). Besides its binding domain to G-actin, profilin interacts with actin related proteins, poly-L-proline (PLP) stretches and phosphatidylinositol-4,5-bisphosphate (PIP2) (reviewed in Jockusch et al., 2007). In mammals, four different profilin isoforms have been described. While all isoforms share similar biochemical properties (Schluter et al., 1997), diverse tissue distribution could be observed. Profilin 1 is ubiquitous, while one of the profilin 2 isoform (PFN2a) is largely restricted to the brain (Witke et al., 2001). Other specific isoforms (PFN2b, PFN3 and PFN4) are present in kidney and testis (Hu et al., 2001;Obermann et al., 2005). Despite their general role in actin dynamic regulation is known, the cell and tissue-specific role of the two isoforms (PFN1 and PFN2a) expressed in the mammalian brain remains largely unknown. A first indication that the two profilins may be involved in mediating synaptic plasticity in cultured hippocampal neurons came from studies which revealed an their activity-

dependent targeting (Neuhoff et al., 2005;Ackermann and Matus, 2003) to dendritic spines. Specifically, electrical stimulation led to a PFN2a accumulation in spine heads and a simultaneous stabilization of the spine structure, whereas blocking the PFN2a targeting into spines destabilized spine structure (Ackermann and Matus, 2003). An additional study in the rat amygdala could emphasize the activity-dependent targeting of profilin to spines of excitatory neurons after fear conditioning (Lamprecht et al., 2006). Interestingly, the brain specific profilin isoform PFN2a interacts with the RhoA downstream kinase ROCK (Witke et al., 1998) and is apparently involved in mediating spine shape changes upon NMDA receptor activation (Schubert et al., 2006). Another study using a PFN2a knock out mouse identified a possible presynaptic function of PFN2a in controlling vesicle exocytosis and presynaptic excitability (Pilo-Boyl P. et al., 2007). In this study however spine morphology and synaptic plasticity were unaltered. Moreover, the role of PFN1 in neuronal morphology was studied in conditional PFN1 knockout mice, where no alteration in the morphology and function of hippocampal neurons could be observed (Gorlich et al., 2012). However, in all these experiments a compensatory effect as for instance an upregulation of PFN1 or PFN2a, respectively, cannot be excluded. Therefore, in a recent study using an acute knock down approach in mature hippocampal neurons a crucial role for PFN2a was described in mediating the actin dependent stabilization of dendrite morphology (Michaelsen et al., 2010). PFN2a deficient pyramidal neurons showed a reduced complexity of dendrites as well as spine number. While overexpressing PFN1 in PFN2a deficient neurons prevented the spine loss, the reduced dendritic complexity could not be restored (Michaelsen et al., 2010).

Taken together, previous studies reveal that two specific profilin isoforms (PFN1 and PFN2a) expressed in the mammalian brain have distinct functions in the fine tuning of neuronal architecture. PFN2a is involved in regulating both, the dendritic structure as well as the spine number and morphology in hippocampal CA1 neurons (Michaelsen et al., 2010;Michaelsen, 2009). However, the detailed cellular mechanisms by which PFN2a exerts this function are still only partially addressed (Michaelsen et al., 2010). Furthermore, whether PFN2a plays a role in maintaining spine stability and is involved in modulating activity-dependent structural plasticity in mature hippocampal neurons remains unknown.

1.6. Aim

Adaptations in neuronal structure as a response to activity generated experience, known as plasticity, are thought to be involved in long term memory storage in the adult brain as well as during developmental processes. However, the cellular and molecular mechanisms underlying the translation of changes in neuronal activity into structural modifications in neurons remain so far elusive. What has to be assumed is that a consecutive transduction from extracellular to intracellular signaling (or vice versa) needs to be involved in processes of synaptic plasticity. Therefore in this thesis, I focused separately on two molecules: the neurotrophin BDNF, known to influence neuronal morphology as an extracellular messenger, and the actin-binding protein profilin2a, known as an intracellular modulator of actin dynamics.

BDNF and its receptor TrkB are involved in many cellular functions including survival, differentiation and activity-dependent synaptic plasticity. In the first part, I was interested in the role of the neurotrophin BDNF and the different signaling pathways activated by its receptor TrkB in mediating the acquisition and maintenance of neuronal morphology. Of special interest here was the analysis of the different consequences of a loss of BDNF in diverse neuronal populations (excitatory and inhibitory neurons).

In the second part, I investigated the involvement of the actin binding molecule profilin2a (PFN2a) in modulating the neuronal architecture of pyramidal neurons in mature organotypic hippocampal slices. Recently, we were in fact able to show by RNAi-mediated knockdown that PFN2a is important for the maintenance of dendritic structure and spine number in mature hippocampal neurons (Michaelsen et al., 2010). In addition, the dendritic structure remodeling upon activity dependent plasticity depends on actin dynamics in the dendrites. Regarding to its role in modulating the actin dynamics, I addressed the question of whether PFN2a might be involved in activity-dependent structural plasticity in mature hippocampal neurons

2. Material and Methods

2.1. Reagents

Agar-Agar	Roth
Agarose	AppliChem
B27 supplement	Gibco
BME Medium	Gibco
Borax	Sigma
Boric acid	Merck
BSA	Roth
CaEDTA	Sigma
Cytosin-D-Arabinofuranosid hydrochloride	Sigma
DAPI	AppliChem
1.1´dioctadecyl-3,3´,3´3´-tetramethylindocarbocyanine-perchlorate (DiI)	Invitrogen
DMEM	PAA
Equine donor serum	HyClone (Perbio)
Fetal calf serum (FCS)	PAA Laboratories
5-Fluoro-2'-Deoxyuridine	Sigma
Fungizon	Gibco
Gelmount	Biomeda
Glucose	Sigma
Glycin	Applicem
Goat Serum	Invitrogen
Hank's Balanced salt solution	Gibco
HBSS	Invitrogen
Kanamycin-sulfate	MP Biomedicals
Kynurenic acid	Sigma

L-Glutamin	Gibco Invitrogen
Lipofectamine 2000®	Invitrogen
Methylenchloride	Sigma
Neurobasal medium	Gibco Invitrogen
Paraformaldehyde	AppliChem
Penicillin/Streptomycin	PAA (P11-010)
Plasmid preparation kit	Qiagen
Poly-L-lysine	Sigma
Polyvinylpyrrolidone (PVP)	Bio-Rad
Spermidine	Sigma
Triton X-100	Sigma
Trypsin-EDTA 1x	Sigma
Tryptone	MP Biomedicals
Uridine	Sigma
Yeast extract	MP Biomedicals

2.2. Solutions and Media

ACSF *(Artificial Cerebrospinal Fluid);*

used for the preparation of acute brain slices and for live imaging experiments.

Component	Molarity [mM]
NaCl	125.0
KCl	2.5
$NaH_2PO4 * H_2O$	1.25
$MgCl_2 * 6H_2O$	2.0
$NaHCO_3$	26.0
D- Glucose	25.0
$CaCl_2 * 2H_2O$	2.0

HBSS used for live imaging experiments.

Component	
HBSS 10x stock solution	50 ml
$CaCl_2 * 2H_2O$	175 mg
$NaHCO_3$	147 mg
H_2O	fill up to 500 ml

Lyses buffer used for the purification of genomic DNA from tails.

Component	Molarity [mM]
Tris/HCl pH 8	100
NaCl	200
EDTA	5
SDS	0.2%
Proteinase K	100µg/ml

2.2.1. Organotypic cultures

Gey's Balanced Salt Solution pH 7.4 (GBSS)

Component	Molarity [mM]
CaCl2 * 2 H2O	1.5
KCl	5
KH2PO4	0.22
$MgCl_2 * 6H_2O$	1.0
$MgSO_4 * 7 H_2O$	0.28
NaCl	1.37
Na_2HCO_3	2.7
Na_2HPO_4	0.86
D-Glucose	5.5

Kynurenic acid

Dissolve 946 mg Kynurenic acid in 5 ml 1 M NaOH, stir 2-3 h, add 45 ml H2Odest., store sterile in 1 ml fractions.

Preparation solution pH 7.2

GBSS	98.0 ml
Glucose	1 ml
Optinal (not for dissociated cultures)	
Kynurenic acid	1 ml

Medium

BME	100 ml
HBSS	50 ml
Equine donor serum	50 ml
L-Glutamin (200mM)	1 ml
Glucose (50%)	1 ml

From DIV 7 on the medium was supplemented with 1.25 µg/ml Fungizone, 100 U Penicillin and 100 µg/ml Streptomycin.

2.2.2. Primary cultures

Medium

Neurobasal	50 ml
B27	1 ml
L-Glutamin (200mM)	125 µl
N2 (100x)	500 µl

Borate-Buffer pH 8.5 (Glass coverslips)

Dissolve 1.24 g boric acid and 1.9 g borax in 400 ml H_2Odest., adjust pH to 8.5.

2.2.3. Immunohistochemistry

Phosphate buffer (0.2 M, pH 7.4)

Component	Molarity [mM]
$NaH_2PO_4*2H_2O$	0.04
$Na_2HPO_4*2H_2O$	0.17

4% PFA in 0.2 M Phosphat Buffer pH 7.4

$H_2O_{dest.}$	500 ml
PFA	40 g
0,2 M Phosphat Buffer	500 ml

Phosphate buffered saline (PBS)

Component	Molarity [mM]
KCl	2.7
KH_2PO_4	1.5
NaCl	137
Na_2HPO_4	10.4

Blocking solution

Component	concentration
BSA	1 %
Goat serum	10 %
Triton-x-100	0,2 %

2.3. Antibodies and DNA

The antibodies used in this thesis are described in Table 1.

Table 1 Antibodies

Antigen	species	manufacturer	dilution
BDNF	mouse	Yves Barde	1:1000
Calbindin	mouse	Swant	1:5000
Calretinin	rabbit	Swant	1:5000
CTIP2	rat	Abcam	1:500
Pavalbumin	rabbit	Swant	1:5000
TrkB-P y705/706	rabbit	Cell signaling	1:1000
Mouse/rabbit/rat Cy2/Cy3/Cy5	goat	Jackson Immuno Res.	1:500

Preparation of DNA

The purification of plasmid DNA for transfection of cultured neurons was done using MAXI or MIDI plasmid purification kits (Qiagen). An overview about the plasmids used in this work is shown in Table 2.

Table 2 Plasmids

plasmid	description	reference
peGFP-f	farnesylated enhanced green fluorescent protein (CMV promoter)	Clontech
pmCherry-f	farnesylated red fluorescent protein mcherry (CMV promoter)	(Shaner et al., 2004; O'Brien, 2007)
pΔCMV-YFP-mPFN2amod.	mouse profilin2a (truncated CMV promoter)	(Boshart et al., 1985;Murk K, 2008)
pRNAT 2.13.iGFPf	polycistronic vector: 1. profilin2a-specific shRNA sequence (CMV/U6.3 promoter), 2. reporter fGFP (CMV promoter)	(Murk K, 2008)
pRNAT 2.13.ΔYFP-mPFN2a mod.	polycistronic vector: 1. profilin2a-specific shRNA sequence (CMV/U6.3 promoter), 2. RNAi-resistant modified profilinIIa (truncated CMV promoter)	(Murk K, 2008)

2.4. Mouse strains

Conditional *bdnf* knockout mice were generated in the group of Prof. Dr. Yves-Alain Barde (University of Basel) (Rauskolb et al., 2010) using the Cre/loP system. The cre recombinase was inserted in the *tau* locus, a gene expressed in postmitotic neurons (Tucker et al., 2001). LoxP sites were inserted and flanked exon IX, the single protein coding exon of *bdnf.* Conditional *bdnf* knockout animals *(cbdnf ko)* were generated by breeding mice carrying two floxed *bdnf* alleles with mice expressing Cre from one allele of the *tau* locus (*tau::cre* line) (Korets-Smith et al., 2004) and also carrying one *bdnf lox* allele. Wild-type animals were *bdnf* $^{lox/lox}$ *tau* ^{wt} (BDNF flox) mice. All experiments with mutant and wild-type animals were performed at postnatal week eight. Genotypes of mice were determined by PCR using a tail biopsy. Wild-type and targeted alleles of the transgenic lines were amplified with specific primer combinations: floxed and wild-type *bdnf* allele (for detailed conditions see Table 3, Table 4, Table 5.
Dissociated neuronal cultures were prepared from *bdnf*$^{lox/lox}$,*tau* ^{wt} mice. Organotypic neuronal cultures were produced from C57BL/6J mice.

2.4.1. Genotyping of transgenic mice

Genomic DNA was extracted from tail pieces. Briefly, tails were digested over night in 500 µl lyses buffer at 55 °C. Cellular debris were removed by centrifugation at 14.000 g. Genomic DNA was extracted using phenol/chloroform and precipitated with ethanol-sodium acetate. Afterwards the DNA was washed once using 70% ethanol and stored at 4 °C in 10 mM Tris/ HCl (pH 8).

Table 3 Primer for genotyping *cbdnf ko* mice

	Primer sequence 5´→ 3´
bdnf 13	GTT GCG TAA GCT GTC TGT GCA CTG TGC
bdnf 14	CAG ACT CAG AGG GCA CTT TGA TGG CTT G
tko forward	CTC AGC ATC CCA CCT GTA AC
tko reverse	CCA GTT GTG TAT GTC CAC CC
cre forward	GCC GAA ATT GCC AGG ATC AG
cre reverse	AGC CAG CAG CTT GCA TGA TC

Table 4 PCR mastermix for 25µl reaction volume

Component	concentration
PCR buffer	10 mM
dNTP´s	10 nM
forward primer	100 nM
reverse primer	100 nM
Taq Polymerase	1 unit
DNA	2 µl

Table 5 PCR protocol for genotyping *cbdnf ko* mice

step		time
1 denaturation	95 °C	2 min
2 denaturation	95 °C	30 sec
3 annealing	61 °C	30 sec
4 synthesis	72 °C	60 sec
5 repeat step	2-4	34 times
6 endsynthesis	72 °C	10 min

PCR products were loaded on a 1.5% agarose gel containing 0.5 µg/ml ethidiumbromide.

2.5. Preparation of Acute cortical brain slices and DiOlistics

For the preparation of the cortical acute brain slices the *cbdnf ko* and wild type mice were briefly anesthetized with CO_2 and rapidly decapitated. After opening the scull the brain was quickly removed and placed into ice-cold carbogenated ACSF for three minutes. To keep the oxidative stress to the neurons as low as possible all following steps were performed in ice-cold carbogenated ACSF. First, the cerebellum was removed with a razor blade und the brain was cut with the VT1200S vibrating microtome (Leica Microsystems, Germany) in 400 µm slices. Therefore, the brain was glued onto the specimen plate and quickly transferred into a buffer-tray filled with ice-cold carbogenated ACSF. Vibratome slices were immediately fixed in 4% PFA overnight at 4 °C.

The labeling of neurons in the cortex with the lipophilic dye DiI was done 24 h postfixation using a hand held gene gun (Bio-Rad; Helios Gene Gun System). The bullets were prepared one day in advance using the tubing preparation station according to manufacturer instructions. Briefly tungsten particles (50 mg; 1.7 µm in diameter; Bio-Rad) were spread on a glass slide, and mixed with 100 µl of dye solution prepared by dissolving 3 mg of DiI (Invitrogen) in 100 µl of methylene chloride (Sigma-Aldrich) (Grutzendler et al., 2003). The dried dye-coated particles were removed from the glass slide, resuspended in 3 ml of distilled water, and sonicated. The dye solution was diluted 1:100 in distilled water. To improve the attachment of the beads to the tube walls, a solution of polyvinylpyrrolidone (PVP, stock solution: 0.05 mg/ml in ethanol) was introduced and after 5 min drained to precoat the tube. Subsequently, the dye solution was filled into the tefzel tubing (Bio-Rad). To spread the dye-coated particles the tube was rotated for 30 min before the liquid was slowly withdraw. The particle-coated tube was dried with a constant nitrogen flow, cut into 13 mm pieces and stored at room temperature for future use.

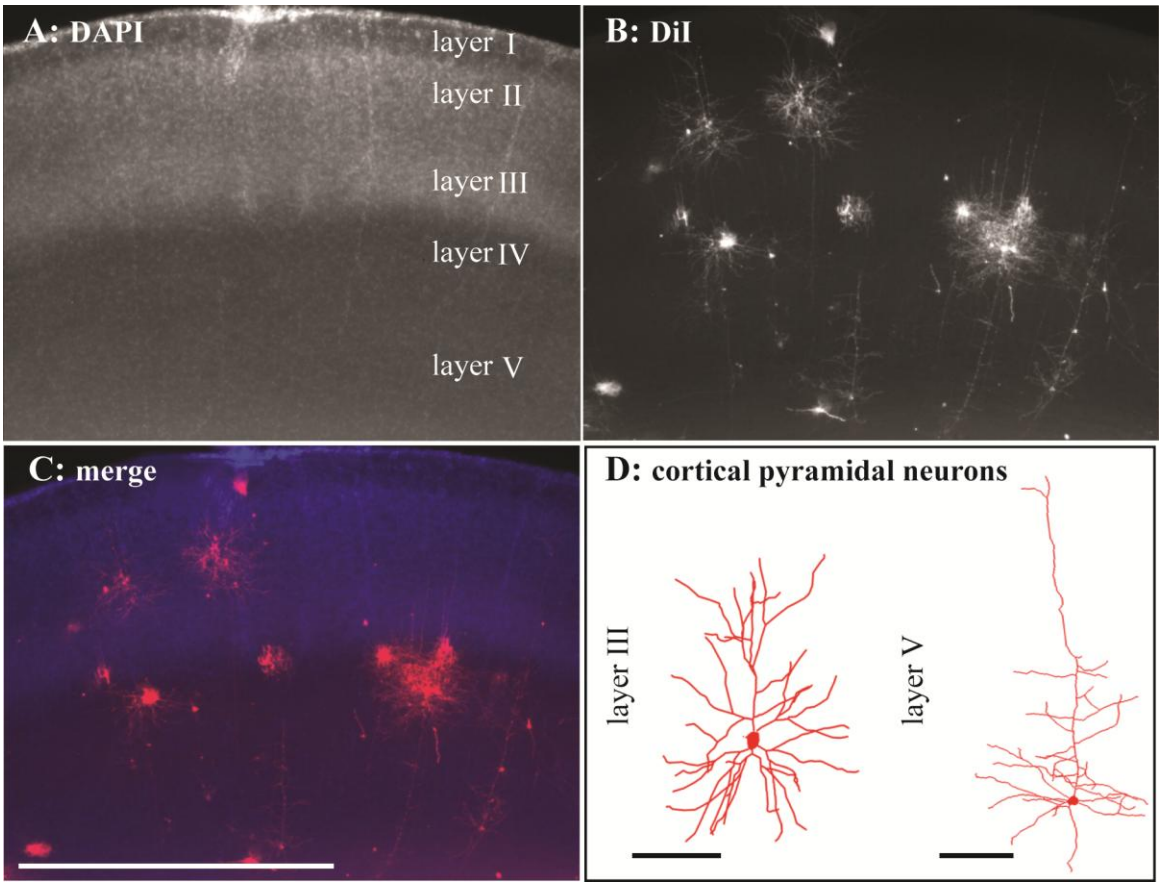

Figure 11: Cortical acute slice labeled with DAPI and DiI of an 8 weeks old mouse.
A: The DAPI staining was used to discriminate the different cortical layer I-V of an acute slice shot with the lipophilic dye DiI **(B)**. **C**: Merged imaged of DAPI and DiI. Scale bar 1000µm. **D:** Typical pyramidal cells of cortical layer III and layer V. Scale bar 100 µm.

DiOlistic

Dye-coated particles were delivered to the acute slices by diOlistic using helium at a pressure of 120 psi. A membrane filter (3 µm; Millipore) was inserted between the barrel aligner and the acute slices to prevent large clusters of particles from damaging the tissue. After shooting, the brain slices were kept in PBS for 5 d at room temperature to allow the dye diffusion. The slices were postfixed with 4% PFA, washed, and stained with DAPI to enable the identification of the different layers in the cortex (Figure 11). Afterwards, the slices were mounted using Fluoro-Gel® (Biomeda).

2.6. Preparation of organotypic hippocampal cultures and Biolistics

Hippocampal slice cultures were prepared and cultivated as previously described (Stoppini et al., 1991). Postnatal day 5 mice (p5; C57/Bl6) were rapidly decapitated, the skull removed and the dorsal half of the brain was transferred into ice cold GBSS. The hippocampi were dissected, cut into 400 µm transversal slices using a McIllwain tissue chopper and kept at 4 °C for 30 min in GBSS. Subsequently, Millicell® cell culture inserts (0.4 µm pore size, Hydrophilic PTFE membrane) were placed in pre-warmed 6-well plates containing 1.1 ml medium per well. 3 slices were plated per insert. After 72 h of cultivation at 36.5°C and 5% CO_2, antimitotics (uridine, cytosine-β-D-arabinofuranoside* hydrochloride and 5-fluoro-2´-deoxyuridine) were applied for 24 h to the cultures to reduce the number of non-neuronal cells. Subsequently, 50% medium was exchanged once a week.

Biolistic gene transfer to cultured hippocampal neurons

Organotypic hippocampal slice cultures were transfected at DIV 7 with fGFP, shPFN2a or shPFN2a/PFN2a using the hand held gene gun.

The bullets for the transfection were prepared one day in advance. For transfections with one plasmid 12.5 mg of 600 nm gold microcarriers (Biorad) and 25 µg of DNA were used, for co-trasnsfections with two plasmids 15 mg of 600 nm gold particles and 30 µg of DNA were prepared. To visualize the neuronal morphology in detail, a membrane targeted form of eGFP (fGFP*)* was transfected at a ratio of 1:1. Gold particles were coated with the respective plasmid by $CaCl_2$ precipitation as follows: Gold particle were mixed with 100 µl of 0.05 M spermidine (Sigma) solution. To prevent aggregation of the

gold microcarriers, the mixed solution was sonicated for 10 s. The plasmids were added to the gold solution. While mixing gently, 100 µl of $CaCl_2$ were added drop-wise followed by 10 min incubation at room temperature. The remaining spermidine was removed by four washing steps with 96% ethanol using a microcentrifuge at 100 g. Finally, the gold micro particles were dissolved in 3 ml 0.05 mg/ml PVP in 96% ethanol. A Tefzel tubing (Biorad) was cleaned and dried for 10 min with a nitrogen flow in the tubing station (Biorad), followed by the insertion of the gold suspension. After 3-5 min the solution was removed. To ensure homogenous distribution of the gold microcarriers, the tubing was rotated 30 s without nitrogen and subsequently dried for 5 min with 3-4 LPM of nitrogen.

Shooting of the different plasmids was done using the hand held gene gun with a helium pressure of 100 psi. To prevent damage of the culture by gold clusters, a membrane filter (3 µm, Millipore) was inserted between the gene gun and the culture.

2.7. Preparation of primary cultures

Preparation of poly-L-lysin coated coverslips

Glass coverslips were incubated in 10 M NaOH for three to five hours at 100°C. After washing five times for 20 min with MilliQ, the coverslips were sterilized at 225°C for six hours and coated with 0.5 mg/ml poly-L-lysine (Sigma) in boric acid buffer (either 2-3 h at 37°C or at 4°C over night). Subsequently, the coverslips were washed with sterile water four to five times, dried and stored at 4 °C.

Preparation of primary cultures

Primary cultures of mouse hippocampal, cortical and striatal neurons were prepared using mice (*bdnf* $^{lox/lox}$; *tau* ^{wt}) at embryonic day E16. Embryos were decapitated and the brains kept in ice cold GBSS/Glucose solution. The hippocampus, striatum and parts of the neocortex were dissected and dissociated separately by 30 min incubation in trypsin/EDTA at 37 °C. The solution was removed and the reaction was stopped by adding 1 ml of DMEM + 2% FCS, followed by mechanical dissociation of the tissue using a fire polished Pasteur pipette. Cells were plated on poly-L-lysine coated glass coverslips and cultured in Neurobasal medium (Gibco) supplemented with 2% BSA

(Gibco), 200 µM L-Glutamin and 1% 100x N2 (Invitrogen) at 37°C, 5% CO_2 and 99% humidity.

Corticostriatal coculture were prepared as described in the study of Penrod and colleagues (2011), except that a ratio of 1:1 (cortex/striatum) was used. The initial cell density was 70000 cells/cm² in a final volume of 500 µl/well. 20% of the medium was exchanged every 7 days.

2.7.1. *bdnf* $^{lox/lox}$*; tau* ^{wt} primary neurons transduced with lentivirus carrying a vector expressing cre recombinase

The expression of the cre recombinase and cGFP as a reporter gen was driven in *bdnf* $^{lox/lox}$; *tau* ^{wt} dissociated neurons using lentiviral infection. Therefore, a lentiviral expression vector with a ubiquitin promoter driven cre recombinase, an IRES (internal ribosomal entry site) sequence and a cGFP reporter was used (UBC-Crig). Two hours after plating the *bdnf* $^{lox/lox}$; *tau* ^{wt} primary neurons, the cells were transduced with the lentivirus UBC-Crig (final concentration 1:10) expressing cre recombinase and thereby inducing the cre mediated gen recombination and deletion of the floxed *bdnf* gen.

2.7.2. Treatments performed on hippocampal primary cultures

BDNF application

Starting from day 2 *in vitro* on (2 DIV) BDNF (40ng/ml) was applied every second day to the medium. Striatal monocultures were fixed at 7 DIV, while hippocampal cultures were treated further at 12 DIV and 17 DIVand finallyfixed at 21 DIV.

Recombinant Human TrkB Fc (TrkB Fc) Chimera application

The TrkB Fc chimera (R&D Systems) (0.5 µg/ml) application was used to scavenge recombinant BDNF and NT4 (Shelton et al., 1995;Ninkina et al., 1997) in primary cultures. The treatment was performed as described for the BDNF application.

CaEDTA application

The Zinc chelator CaEDTA (1.5mM) (Huang et al., 2008;Frederickson et al., 2002) was repeatedly applied to the medium either starting on 1 DIV, 7 DIV or 14 DIV every 7 days.

2.7.3. Transfection of primary neurons

Primary neurons were transfected using Lipofectamine2000® at 20 DIV, except for striatal monocultures which were transfected at 6 DIV. Half of the medium was exchanged for Neurobasal medium without supplements and the old medium was stored at 36.5°C and 5% CO_2. For the transfection, 0.8 µg DNA and 2 µl Lipofectamine per well were diluted separately in 50 µl of Neurobasal medium, both solutions were combined after 5 min and further incubated for 20 min at room temperature. 100 µl transfection solution were added to each well drop wise followed by an incubation for 45 min at 36.5°C and 5% CO_2. Afterwards, the medium was replaced by the previously collected old cell culture medium.

2.7.4. Immunohistochemistry

Primary neurons were fixed using 4% PFA at 4 °C for 15 min, followed by three washing steps with PBS for 20 min. Blocking of unspecific binding sites was performed for 1 h at room temperature in a BSA blocking solution. Afterwards, primary antibodies were diluted in blocking solution (for antibody concentration see Table 1) and incubated over night at 4°C. The secondary antibodies were diluted in PBS and incubated for 2 h at room temperature. After washing with PBS coverslips were mounted upside down in Fluoro-Gel®.

2.8. Imaging

Neurons in acute slices were imaged with an Axioplan 2 imaging microscope (Zeiss) equipped with an ApoTome (Zeiss). Each neuron of cortical acute slices was first imaged with a 20x (0.8 N.A.) objective (Zeiss) with a z-sectioning of 1 µm. In primary cultures plain fluorescence images of neurons were acquired. Morphological reconstruction of the neurons and their processes was achieved using Neurolucida software (MicroBrightField) Subsequently, Sholl analysis (SHOLL, 1953) was performed using the Neuroexplorer software (MicroBrightField). Briefly, for Sholl analysis the program determines the number of dendritic intersections for gradually increasing concentric circles centered at the cell body (Figure 12). Spine density of primary neurons was imaged using higher magnification images (63x 1.4 N.A.,Zeiss) and a z-stack thickness of 0.5 µm. For analysis of spine density and dendritic spine types in cortical pyramidal neurons of acute brain slices images were acquired with a LSM510 Meta confocal microscope (Zeiss) using a 40x (0.8 N.A.) water-immersion objective with a zoom 4 and were z-sectioned at 0.3 µm. The number of spines was determined per micrometer of dendritic length. For the analysis of dendritic spine types, the Neurolucida software was used to measure length, as well as head and neck diameter of each spine. The three measurements were used to fit each spine within the three classical spine types: stubby (type I), mushroom (type II) and thin (type III) (Harris et al., 1992).

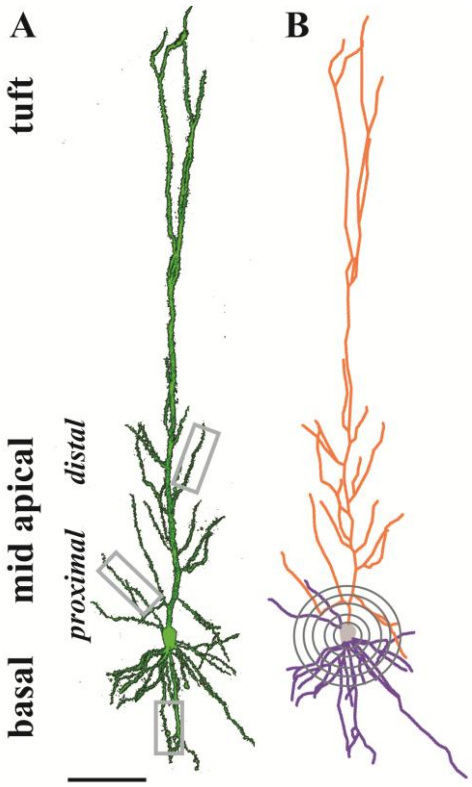

Figure 12 Morphological analysis of neurons
A: GFP expressing CA1 pyramidal neuron captured from a maximum intensity projection. The squares are indicating the different regions for spine density counting and spine types identification. Scale bar, 100µm. **B:** Neurolucida representation (tracing) of the the CA1 pyramidal neuron shown in A. The circles are indicating the Sholl analysisi performed with Nuerolucida Explorer.

Live imaging experiments

Live imaging experiments at different time points (1, 5, 7 and 9 DIV) post-transfection were used to document dendritic alterations upon transfection of organotypic hippocampal cultures with different expression vectors.

The organotypic hippocampal cultures were transferred into modified HBSS (Lang et al., 2007) supplemented with Fungizone (1.25 µg/mL, Gibco), penicillin (10,000 U/mL), and streptomycin (10 mg/ml). Imaging was performed using an upright Olympus Cell^M imaging system controlled by the Cell^M software and equipped either with a 20x objective (0.8 N.A.) for dendrite analysis or a 40x objective (0.8 N.A.) for spine analysis (Olympus). After image acquisition (1, 5, 7, and 9 DIV), the cultures were transferred back into the incubator in culture medium containing the same antibiotics as above.

To further analyze the spine dynamics under basal conditions and spine shape changes upon induction of chemical long-term potentiation (10 mM Glycine for 10 min) (Shahi et al., 1993), organotypic hippocampal cultures were imaged at 9 DIV post-transfection in ACSF using an Olympus Fluoview1000 microscope (60x, 1 N.A.). To assess the spine shape changes before (baseline) and after chemical long-term potentiation induction the selected dendritic segment was imaged 60 min before and 60 min after chemical long-term potentiation induction. Spine dynamics were determined by imaging the same stretch of dendrite for 20 min every 5 min. Dendritic spine length and head diameter were measured for all spines identified on a defined dendritic stretches using ImageJ.

The statistical analysis for the Sholl analysis, spine density and spine type distribution, as well as spine dynamics, was performed in Excel (Microsoft) and GraphPad Prism 4 (GraphPad). All data shown are presented as mean ± SEM. The data obtained were compared between two different experimental conditions using a two-tailed Student *t* test assuming equal variance. Asterisks indicate the significance levels as follows: **p*=0.05, ***p*=0.01, and ****p*=0.001.

3. Results

3.1. Role of BDNF in regulating the dendritic structure of excitatory and inhibitory neurons

Much is known about the role of Brain-Derived Neurotrophic factor (BDNF) in regulating neuronal differentiation, synaptic plasticity as well as dendritic arborization (Huang and Reichardt, 2001). However, as a *bdnf* null mutation leads to death soon after birth, the BDNF role in modulating the neuronal structure during central nervous system (CNS) postnatal development was so far difficult to assess *in vivo*. A new conditional mouse mutant *(cbdnf ko)* in which BDNF is lacking specifically in neurons throughout the CNS revealed a surprisingly area specific requirement for BDNF in regulating dendritic structure (Rauskolb et al., 2010). Indeed, the analysis of the length and complexity of dendrites and of dendritic spine density in mature inhibitory medium spiny neurons (MSNs) of the striatum show a highly significant reduction in *cbdnf ko* compared to wild type mice. On the contrary, the analysis of the morphology of mature excitatory CA1 pyramidal neurons in *cbdnf ko* reveal only minimal effects on dendritic length, complexity and spine density. The only alteration observed in the pyramidal neurons in the hippocampus of *cbdnf ko* mice is a significant decrease in the proportion of mushroom-type spines (Rauskolb et al., 2010;Dreznjak, 2008).

The results described above leave two possible interpretations open: 1) that the effect of BDNF on dendritic architecture could be area-specific (hippocampus vs. striatum) or 2) that it could be cell type-specific (excitatory pyramidal neurons vs. inhibitory MSNs). The aim of the first part of my work was to discriminate between a possible area-specific and a cell type-specific role of BDNF by comparing the dendritic architecture of excitatory and inhibitory neurons in different brain areas both *in vivo* in *cbdnf ko* and wild type (wt) mice and in a BDNF depleted neuronal culture system. Furthermore, I addressed the molecular mechanism mediating the specific effect of BDNF on the morphology of different neuronal populations.

3.1.1. Minimal morphological changes in excitatory cortical pyramidal neurons in *cbdnf ko* mice *in vivo*

To further analyze the area-specific requirements for BDNF during postnatal development, the dendritic morphology of single excitatory pyramidal neurons in

different cerebral cortex layers was investigated in cortical acute slices of 8 weeks old *cbdnf ko* mice. In *cbdnf ko* mice the volume of the cerebral cortex showed a reduction of about 20% compared to the one of wild type mice (Rauskolb, 2008). A DiOlistic approach was used to perform a detailed analysis of completely labeled dendrites, including dendritic spines, of isolated pyramidal neurons in both layer II/III and layer V of the cortex. DiI labeled pyramidal neurons in layer II/III and layer V were identified by the pyramidal shaped cell body and by the presence of two distinct dendritic trees, one long apical dendrite and several short basal dendrites. The classification of the pyramidal cells in the different layers was done using a DAPI staining to identify the six cortical layers. Due to the slight differences in their dendritic architecture layer II/III and layer V pyramidal neurons were analyzed separately. To quantify the dendritic complexity using a Sholl analysis (SHOLL, 1953), apical and basal dendrites were analyzed separately due to their different morphology and connectivity. Compared to wild type (wt) neurons the total dendritic complexity of *cbdnf ko* layer II/III pyramidal neurons was slight reduced (Figure 13A´´) due to a significantly lower complexity in a limited distal portion of their apical dendrite. No alteration could be observed for the total dendritic complexity and for the Sholl analysis of basal dendrites of layer II/III cells in *cbdnf ko* (Figure 13A´´). The Sholl analysis of *cbdnf ko* layer V pyramidal neurons revealed no differences in dendritic complexity both of the apical and basal dendritic tree compared to control neurons (Figure 13B´´). In the next step spine density was counted and the spine type distribution (Harris et al., 1992) was analyzed from the mid apical part of apical dendrites. *Cbdnf ko* pyramidal neurons of both layers showed no significant changes in their spine density (Figure 13C´´), while significant alterations in spine type distribution of both layers (pooled data) could be observed. The spines were classified as follows: stubby spines are shorter than 1 µm, whereas the mushroom spine shows a bulbous head which can be clearly discriminated from the narrower neck. Thin spines are longer than 1 µm and carry only a small head (Harris et al., 1992). In *cbdnf ko* neurons the proportion of mushroom spines was significantly reduced, whereas the one of thin spines was significantly increased (Figure 13D). The very mild or absent effect of BDNF depletion on dendritic complexity and dendritic spine density respectively associated to a shift in the proportion of mushroom and thin spines observed in *cbdnf ko* cortical pyramidal neurons perfectly reproduces what we previously observed in the hippocampus of 8 weeks old *cbdnf ko* mice (Rauskolb et al., 2010). These observations strongly suggest that BDNF is not

required for dendritic growth of hippocampal CA1 and cortical pyramidal neurons, but only for their spine type distribution.

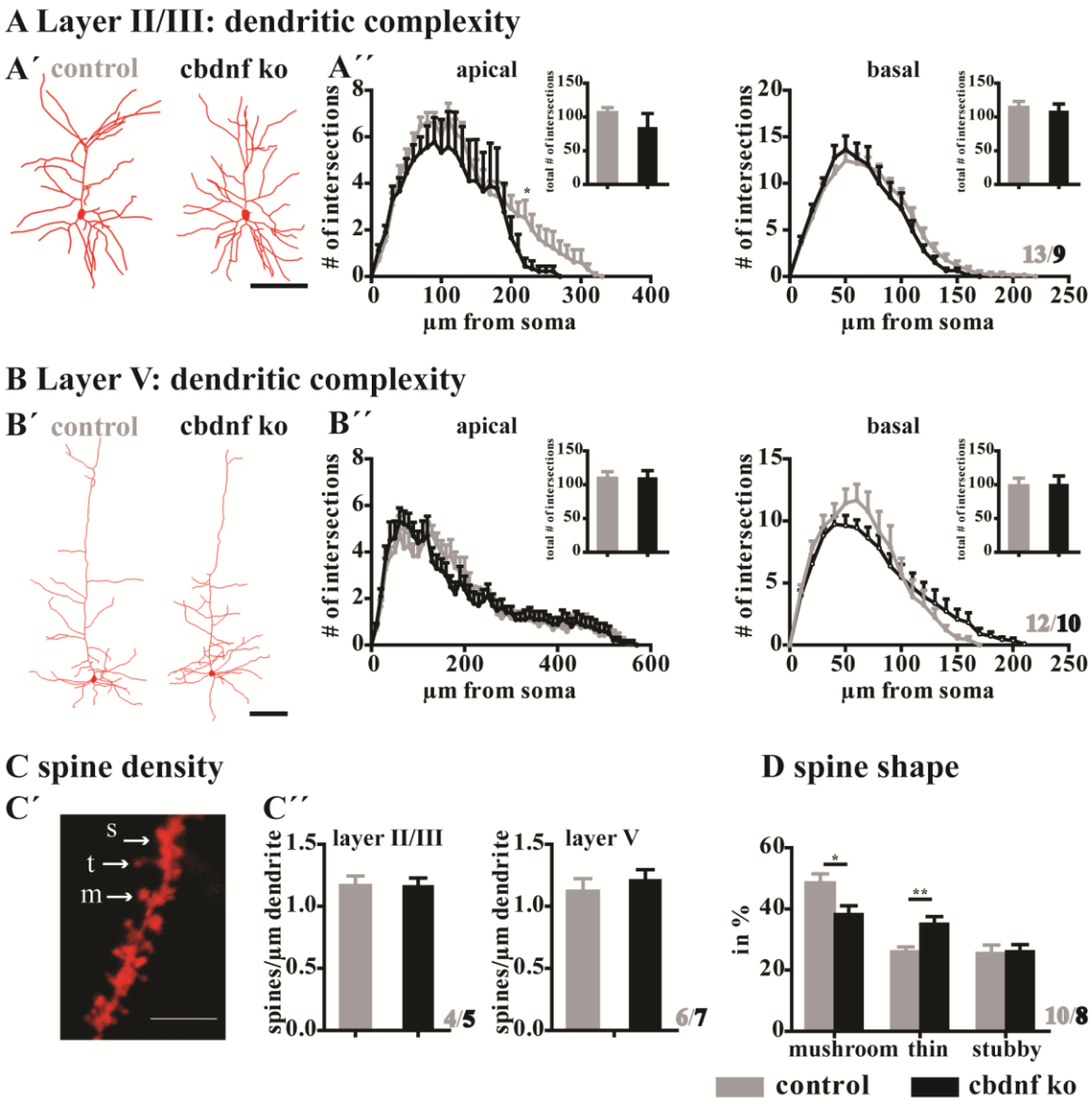

Figure 13 Minimal morphological effects in excitatory pyramidal neurons of cerebral cortical layer II/III and layer V in 8 weeks old *cbdnf ko* mice

A: Dendritic complexity of pyramidal neurons in layer II/III was minimal changed in *cbdnf ko* mice compared to control mice. **A´:** Images of morphology of control and *cbdnf ko* pyramidal neuron in layer II/III. Scale bar 100µm. **A´´:** Sholl analysis and the total dendritic complexity (inserted graph) of the apical and basal dendrites of layer II/III pyramidal neurons in *cbdnf ko* and control mice. **B:** No changes in dendritic complexity of layer V pyramidal neurons in *cbdnf ko* mice. **B´:** Images of morphology of control and *cbdnf ko* pyramidal neurons in layer V. Scale bar 100µm **B´´:** The Sholl analysis and the total dendritic complexity (inserted graph) of the apical and basal part of pyramidal neurons in layer V. **C:** Spine density was unchanged in the apical part of cortical neurons in layer II/III and layer V in *cbdnf ko* mice, while mushroom spine types were significant reduced and thin spines increased in *cbdnf ko* mice **(D)**. **C´:** Stretch of DiI labeled apical dendrite of a pyramidal neuron in layer II/III showing the different spine types. (s) stubby, (t) thin, (m) mushroom. **C´´:** spine density of layer II/III and layer IV pyramidal neurons. **D:** Spine type distribution of the apical dendrite of both cortical layers. **p*:0.05, ***p*:0.01.

Taken together, the data from the analysis of the *cbdnf ko* mice show that the neuronal morphology of inhibitory medium spiny neurons is strongly impaired after BDNF loss, while excitatory neurons in the hippocampus and cortex reveal only minimal changes in their neuronal morphology. To investigate why different neuronal populations respond in a different way to the loss of BDNF, an *in vitro* system with primary neuronal cultures of *bdnf* $^{lox/lox}$ *; tau* ^{wt} mice infected with a lentivirus expressing cre recombinase was used.

3.2. BDNF knockdown in *bdnf* $^{lox/lox}$ *; tau* ^{wt} primary hippocampal neurons

Before the primary cultures could be used to investigate further morphological effects and the mechanism in response to loss of BDNF, the system needs to be tested, including the viral mediated deletion of the floxed *bdnf* gene and whether the different *in vivo* morphological phenotypes (Rauskolb et al., 2010) can be reproduced *in vitro*. Neurons in the *cbdnf ko* mice were analyzed after 8 weeks to assess the regulating role of BDNF. To be consistent with the *in vivo* conditions, all neurons in primary cultures were analyzed at 21DIV to investigate the long-term effect of BDNF, too

3.2.1. BDNF detection

Mature primary hippocampal neurons infected with the cre-encoded virus were used to detect the BDNF expression levels. Therefore an immunhistochemistry using a specific antibody against BDNF was performed. The successful viral tranduction of neurons could be visualized *via* the cGFP expression (Figure 14B). Infected *bdnf* $^{lox/lox}$ *; tau* ^{wt} cells showed a reduced BDNF (Figure 14B´´) signal compared to *bdnf* $^{lox/lox}$ *; tau* ^{wt} neurons without the cre recombination (Figure 14A´).

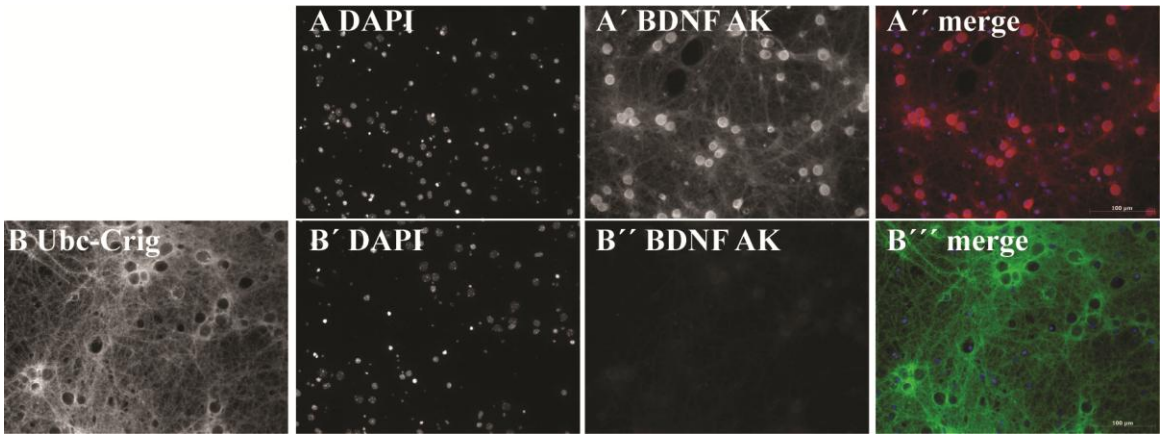

Figure 14 BDNF detection in 21DIV old primary hippocampal cultures of *bdnf* $^{lox/lox}$ *; tau* ^{wt} mice upon cre mediated recombination
A: *bdnf* $^{lox/lox}$ *; tau* ^{wt} neurons without virus infection. Almost all DAPI **(A)** positive cells showed a positive BDNF antibody signal **(A´, A´´)**. **B:** *bdnf* $^{lox/lox}$ *; tau* ^{wt} neurons after cre induced recombination. Most of the DAPI positive cells **(B´)** were infected with the virus, visualized with cGFP expression **(B, B´´´)**. BDNF antibody staining showed a reduced signal after cre-induced recombination **(B´´)**. Scale bar 100 µm.

3.2.2. Neuronal morphology of inhibitory medium spiny neurons *in vitro* after BDNF depletion

The loss of BDNF in *cbdnf ko* mice revealed the strongest phenotype in the morphology of mature inhibitory GABAergic medium spiny neurons (MSNs) in the striatum with reduced dendritic complexity (Figure 15A) and spine number (Rauskolb et al., 2010). To examine if this strong *in vivo* phenotype can be reproduced *in vitro* in the cell culture system, mature (21DIV) MSNs from *bdnf* $^{lox/lox}$*; tau* ^{wt} mice were prepared and infected with the cre-encoded virus at the time of plating. To mimic the *in vivo* situation where the striatum receives both excitatory afferent activity and BDNF from the cortex, a corticostriatal coculture system was used (Penrod et al., 2011). To visualize the neurons the cultures were transfected with an expression plasmid for fCherry using the Lipofectamin2000 system. Mature MSNs were identified using immunohistochemistry against Ctip2, a transcriptional factor that regulates the differentiation of MSNs (Arlotta et al., 2008). The Sholl analysis with plotting the number of intersections against the distance from the cell body was used to determine the dendritic complexity.

The strong phenotype observed regarding the morphology of neurons in the striatum *in vivo* could be reproduced *in vitro* (Figure 15). The Sholl analysis revealed a significantly reduced dendritic complexity of mature MSNs in the absence of BDNF (BDNFΔ: corticostriatal coculture expressing the cre recombinase) compared to control MSNs (Figure 15B).

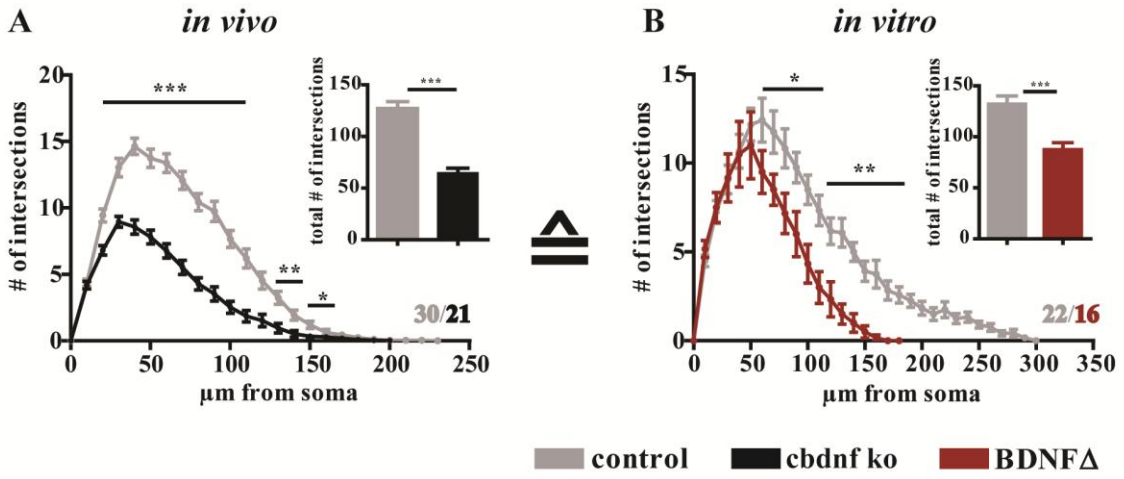

Figure 15 Neuronal morphology of inhibitory medium spiny neurons *in vitro* is reduced after BDNF depletion
A: Sholl analysis and total dendritic complexity (inserted graph) of MSNs showed significantly reduced dendritic complexity in 8 weeks old *cbdnf ko* mice *in vivo* (Rauskolb et al., 2010). **B:** Sholl analysis and total dendritic complexity (inserted graph) of mature (21DIV) MSNs *(in vitro)* of BDNF depleted primary corticostriatal cocultures (BDNFΔ) revealed a significant reduction in their dendritic complexity compared to control MSNs (without cre viral infection) as well. **p*:0.05, ***p*:0.01, and ****p*:0.001.

3.2.3. Neuronal morphology of hippocampal neurons *in vitro* after BDNF depletion

The excitatory hippocampal CA1 pyramidal neurons showed a mild reduction in their dendritic complexity in 8 weeks old *cbdnf ko* mice (Figure 17A´). While the spine number of CA1 neurons was not altered (Figure 17B´), the spine types showed a significant reduction in mushroom spines and increase in thin spines in *cbdnf ko* mice *in vivo* (Figure 17B´) (Rauskolb et al., 2010). The same phenotype could be observed in the morphology of mature hippocampal neurons *in vitro* (DIV21) of BDNF depleted neurons by cre reconbinase expression (BDNFΔ). BDNF depleted neurons could be detected by their cGFP expression and visualized via the expression of fCherry (Figure 16). While the dendritic complexity (Figure 17A´´) and spine number (Figure 17B´´) were not affected by the loss of BDNF, the spine type distribution (Figure 17B´´) was significantly different in BDNF depleted hippocampal neurons compared to control neurons. The spine types were discriminated as described from Harris and colleagues (1992). The mushroom spine types were significantly reduced and thin spines increased in BDNF depleted hippocampal neurons compared with control neurons without cre recombinase induced BDNF deletion (Figure 17B´´).

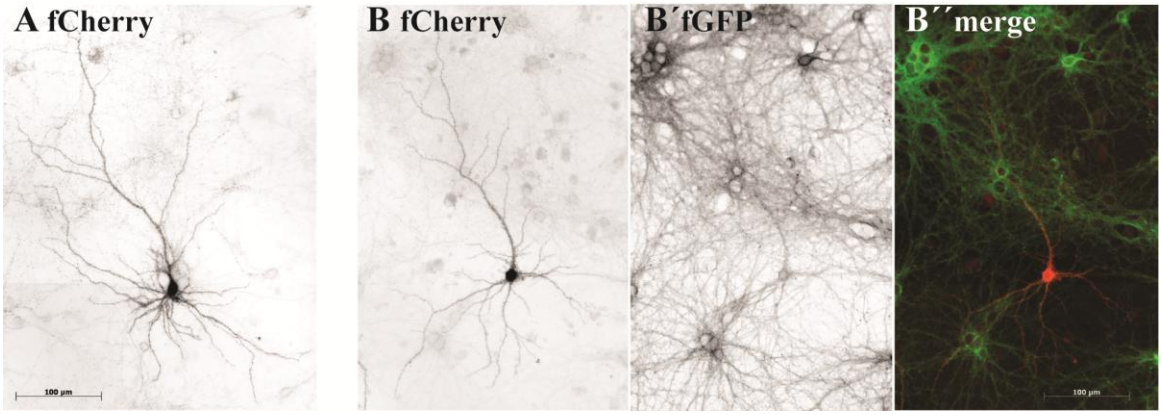

Figure 16 Mature hippocampal neurons of control and BDNF depleted primary cultures
A: typical control hippocampal neuron expressing fCherry. **B:** fCherry expressing primary hippocampal neuron which underwent cre mediated recombination, visualized with cGFP **(B´, B´´)**. Scale bar 100µm.

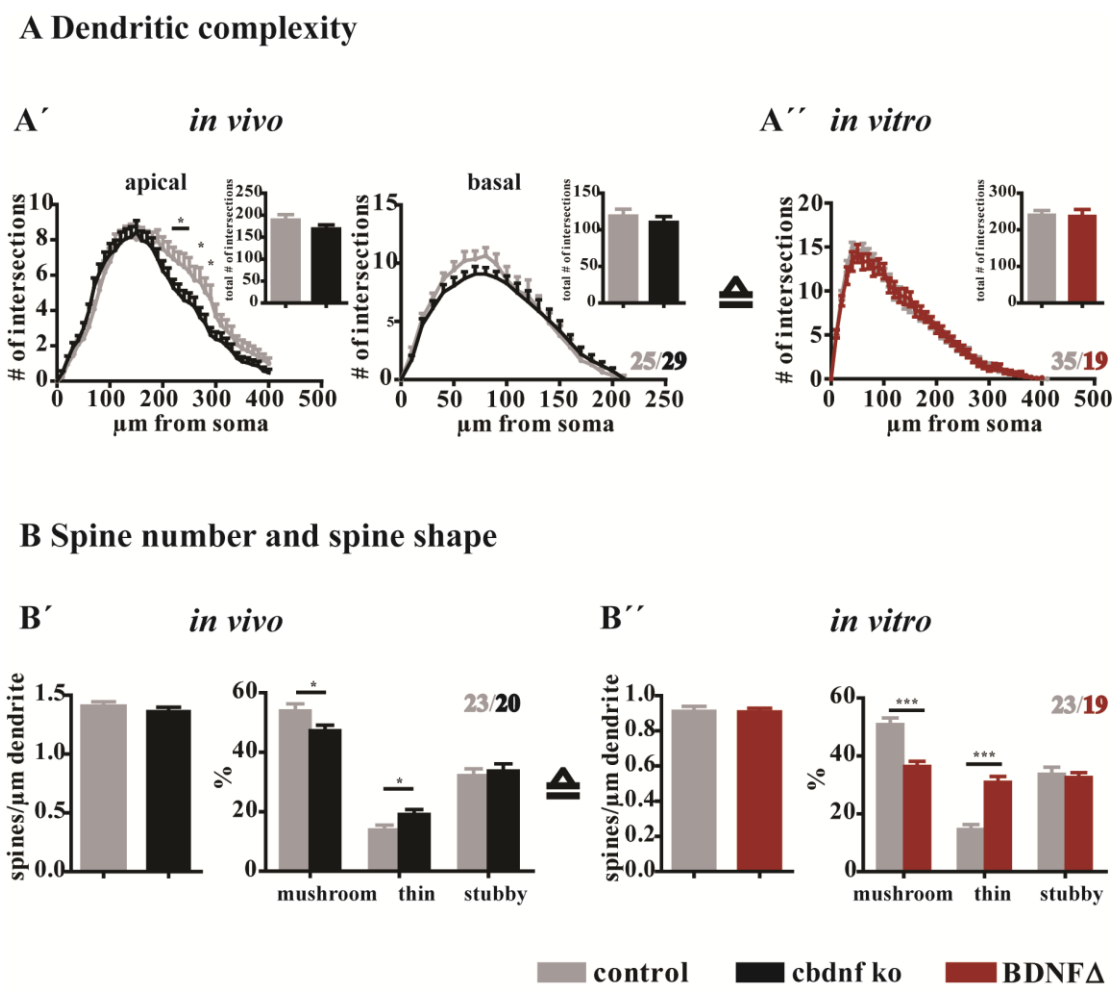

Figure 17 Neuronal morphology of BDNF depleted hippocampal neurons
A: Sholl analysis and total dendritic complexity (inserted graph) of hippocampal pyramidal neurons of 8 weeks old *cbdnf ko* mice (A´) *(in vivo)* as well as pyramidal neurons (A´´) *(in vitro)* of BDNF depleted primary hippocampal culture mice were essentially unchanged compared to the respectively control **B:** spine density was not altered while spine types distribution was significantly different in *cbdnf ko* hippocampal CA1 neurons (B´) and mature pyramidal neurons of BDNF depleted (B´´) primary hippocampal culture. **p*:0.05 and ****p*:0.001.

In this set of data we could show that BDNF depletion could be achieved upon expression of the cre recombinase in *bdnf* $^{lox/lox}$ *; tau* ^{wt} primary neuronal cultures and that the phenotypes observed *in vivo* both in the striatum and the hippocampus could be reproduced *in vitro*. Taken together, these results confirm that primary neuronal cultures can be used to further analyze the molecular mechanisms mediating the role of BDNF in regulating neuronal morphology of different cell types.

3.3. BDNF plays a crucial role in regulating the morphology of cortical and hippocampal inhibitory neurons

In adult *cbdnf ko* mice, BDNF is required for the postnatal dendritic growth of the inhibitory GABAergic medium spiny neurons (MSNs) in the striatum (Rauskolb et al., 2010). This is in agreement with previous reports showing that BDNF plays a crucial role in development of GABAergic striatal neurons (Mizuno et al., 1994). In addition, BDNF has been shown to promote as well the differentiation of GABAergic neurons in the hippocampus and cortex (Vicario-Abejon et al., 1998;Kohara et al., 2003). Therefore, I next analyzed the morphology of mature inhibitory neurons in the cortex and hippocampus to address the question of whether GABAergic neurons were affected by BDNF depletion in the same areas where *cbdnf ko* excitatory neurons revealed only a mild phenotype. The aim of these experiments was to be able to dissociate an area-specific from a cell type-specific effect of BDNF in regulating the postnatal dendritic growth.

The morphology of mature inhibitory neurons of the cortex and hippocampus was analyzed in primary cultures following BDNF knockout In BDNF depleted primary hippocampal and cortical cultures, different GABAergic interneurons subtypes were analyzed at 21DIV using an immunhistochemistry against three calcium binding proteins, parvalbumin, calretinin or calbindin. In positive interneurons these calcium binding proteins are distributed all over the cell making it possible to use the immunhistochemical labeling to trace the dendrites. The dendritic complexity of single inhibitory neurons in cortical or hippocampal BDNF depleted cultures was analyzed separately by Sholl analysis and compared to the one of control inhibitory neurons. All three types of GABAergic interneurons showed a significant reduction in their total dendritic complexity in both, the cortical and hippocampal BDNF depleted (BDNFΔ) primary neurons compared to control inhibitory neurons (Figure 18). The Sholl analysis could

reveal a significant reduction in their dendritic complexity more proximal to the cell body. These results suggest a crucial role for BDNF in modulating the dendritic architecture of GABAergic inhibitory neurons not only in the striatum, but also in the cortex and hippocampus. Although the morphology of mature excitatory neurons in the cortex and hippocampus revealed a mild phenotype, the dendritic complexity of mature inhibitory neurons within the same brain areas showed a significant reduction in the absence of BDNF. These results strongly indicate that the effect of BDNF in regulating dendritic growth occur cell type-specific rather than in an area-specific way.

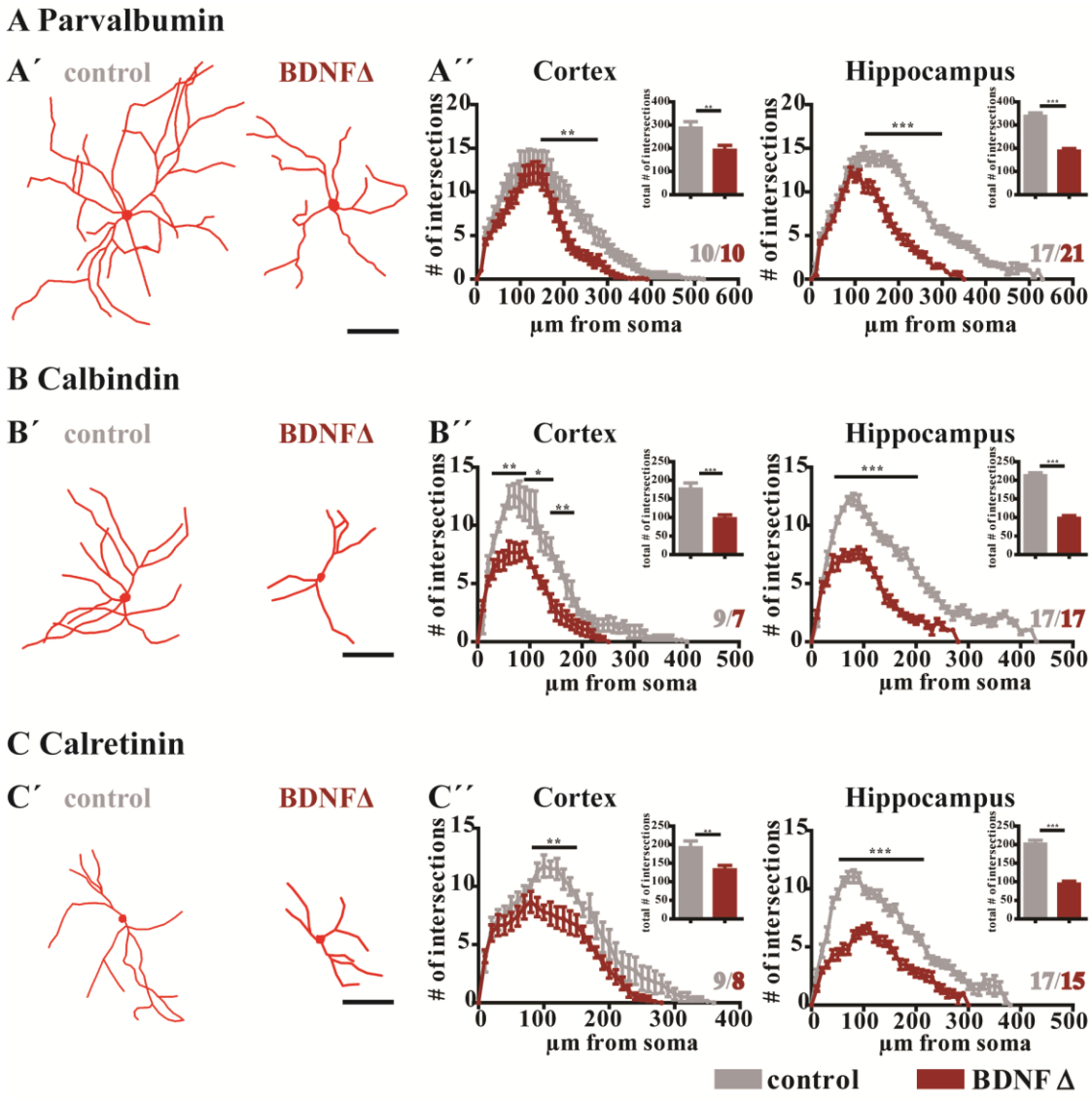

Figure 18 Reduced dendritic complexity of parvalbumin, calbindin, calretinin expressing interneurons in hippocampal and cortical BDNF depleted cultures
A, B, C: Parvalbumin, calbindin or calretinin expressing interneurons showed a significantly reduced dendritic complexity in the cortical and hippocampal BDNF depleted primary cultures. **A´, B´, C´:** Images of the morphology of the respectively control and BDNFΔ interneuron in the hippocampus**. A´´, B´´. C´´:** Sholl analysis and total dendritic complexity (inserted graph) of BDNF depleted parvalbumin, calbindin or calretinin expressing interneurons in the cortical and hippocampal primary cultures compared with control interneurons. ***p*:0.01 and ****p*:0.001.

3.4. Altered TrkB phosphorylation in inhibitory but not excitatory neurons after BDNF depletion

While, upon the loss of BDNF, both in *cbdnf ko* mice and in BDNF depleted primary cultures excitatory neurons from different brain areas revealed just mild changes in their neuronal morphology, inhibitory neurons showed a highly significant reduction of their dendritic complexity. Due to its role as positive regulator of neuronal morphology (Lu et al., 2005), it is likely that the BDNF receptor TrkB might still be able to modulate the dendritic structure of excitatory neurons even in the absence of BDNF. Upon ligand binding, the TrkB receptor gets phosphorylated and then activates a series of intracellular pathways mediating its effects (reviewed in Chao, 2003). Therefore, the first experiment I performed was to analyze the levels of TrkB receptor phosphorylation in mature inhibitory (MSNs) and excitatory neurons (hippocampal pyramidal cells) upon cre recombinase mediated BDNF deletion in *bdnf* $^{lox/lox}$ *; tau* ^{wt} primary cultures. To this aim I performed immunhistochemistry with an antibody against the TrkB phosphorylation site Y705/706. Neurons in which the Ctip2 (transcription factor in MSNs) and TrkB Y705/706 antibody staining were colocalized were used to analyze the phosphorylation levels of TrkB in inhibitory striatal MSNs in the corticostriatal cocultures. Primary neurons without BDNF deletion were used to determine the TrkB phosphorylation level under normal culture conditions. The antibody staining against the TrkB phosphorylation site Y705/706 showed a reduced signal in inhibitory MSNs upon BDNF deletions (BDNFΔ) in corticostriatal primary coculture compared to control MSNs (Figure 19A).

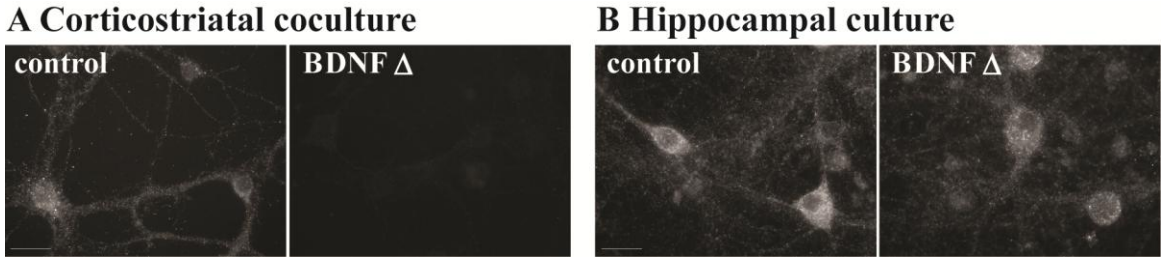

Figure 19 Different TrkB phosphorylation in inhibitory and excitatory BDNF depleted neurons
A: Antibody staining against the TrkB phosphorylation site Y705/706 revealed a reduced signal in inhibitory MSNs after BDNF depletion (BDNFΔ) compared to neurons without BDNF depletion (control), whereas the antibody signal in excitatory hippocampal neurons was not altered in BDNF depleted dissociated neurons **(B)**.

Remarkably, the levels of TrkB phosphorylation were not different in excitatory hippocampal control and BDNF depleted (BDNFΔ) primary cultures (Figure 19B). Thus, despite BDNF deletion, the BDNF receptor TrkB is still phosphorylated in mature

hippocampal excitatory neurons but not in striatal inhibitory neurons. These data show that the TrkB receptor is normally phosphorylated even after removing its main ligand BDNF.

3.5. BDNF application rescues spine type phenotype in *cbdnf ko* primary hippocampal neurons

When compared to controls the hippocampal pyramidal neurons in the CA1 region in 8 weeks old *cbdnf ko* mice as well as the mature hippocampal neurons in BDNF depleted primary neurons exhibited a reduced mushroom spine type and increased thin spine type as the only significant change. In the next set of experiments I assessed whether the spine shape phenotype in the BDNF depleted mature hippocampal neurons can be rescued by applying BDNF. Therefore BDNF (40 ng/ml) was applied to the medium at 2, 4, 6, 12 and 17 DIV of hippocampal primary cultures of *bdnf* $^{lox/lox}$ *; tau* ^{wt} mice infected or not with a cre encoded virus. BDNF was in a solution containing in BSA to prevent its sticking to the tissue. Therefore, as a control only BSA was added to cultures in the same amount as for BDNF. As no significant difference between non-treated and BSA treated cultures could be observed, the results were combined. The dendritic complexity of mature hippocampal neurons was indistinguishable between the control, BDNF depleted (BDNFΔ) and BDNF depleted neurons after BDNF application (BDNFΔ+BDNF) (Figure 20A). Longer and significantly more complex dendrites could be observed after BDNF application to control hippocampal cultures (+BDNF) (Figure 20A). In addition, spine density was not altered in BDNFΔ+BDNF neurons or after applying BDNF to control cultures compared to non-treated cells (Figure 20B). Interestingly, the addition of BNDF to BDNF depleted hippocampal neurons could rescue the spine type distribution of BDNF depleted cells to the levels seen in control cells. Mushroom spine types were significantly increased, while the thin spines showed a significant reduction in BDNFΔ+BDNF hippocampal neurons compared to BDNF depleted neurons. The addition of BDNF to control hippocampal neurons had no effect on their spine type distribution (Figure 20C´´). These results showed that the significant different spine type distributions in BDNF depleted hippocampal neurons could be rescued by applying BDNF. No additional effects after application of BDNF to control neurons could be observed in spine density as well as spine type distributions, except the elongation and increased complexity of dendrites after BDNF addition to control neurons.

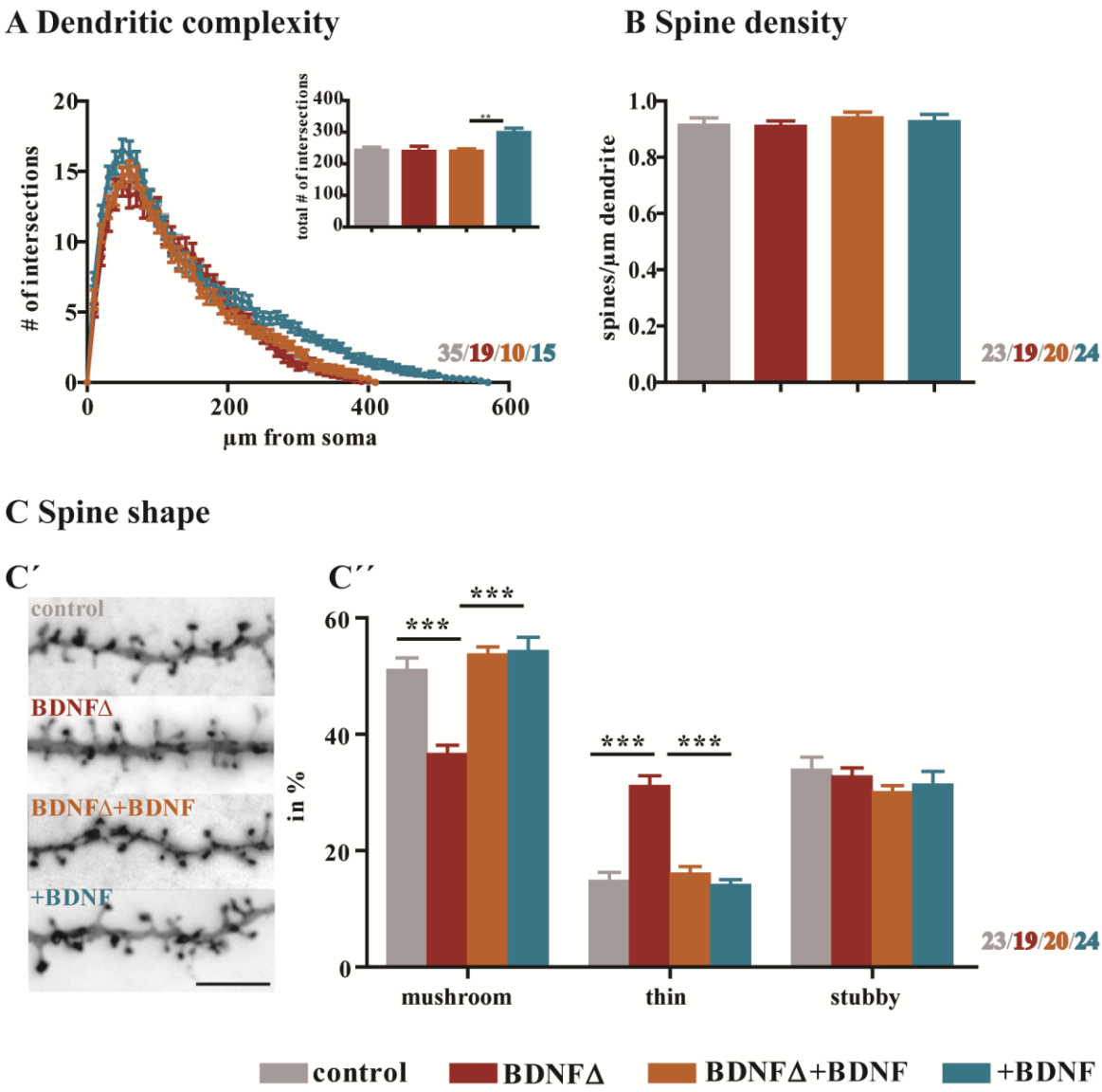

Figure 20 Rescue of the spine phenotype in BDNF depleted hippocampal neurons by BDNF application

BDNF (40ng/ml) was added to cultures at 2, 4, 6, 12 and 17DIV. **A:** Sholl analysis and total dendritic complexity (inserted graph) of control, BDNF depleted (BDNFΔ) and BDNF depleted with BDNF treated (BDNFΔ+BDNF) neurons showed no difference. Applying BDNF to control neurons (+BDNF) resulted in elongation of dendrites and increased total dendritic length. **B:** spine density was indistinguishable between all treatments. **C:** Deceased mushroom spine types and increased thin spines in BDNF depleted neurons could be rescued by applying BDNF. The addition of BDNF to control neurons revealed no changes in the spine type distribution compared to non-treated control neurons. **C´:** spine images of hippocampal neurons with the different treatments. **C´´:** Spine types distribution in hippocampal neurons after different treatments. ***p:0.001.

3.6. NT4 is not involved in maintaining the neuronal structure of hippocampal neurons in the absence of BDNF

The next sets of experiments were aimed at understanding the mechanism mediating the activation of the TrkB receptor in the absence of its ligand BDNF thereby possibly modulating the neural morphology of hippocampal neurons in a BDNF independent way. BDNF is not the only neurotrophin binding to TrkB. It is known that the neurotrophin 4 (NT4) can activate the TrkB receptor as well (Chao, 2003).

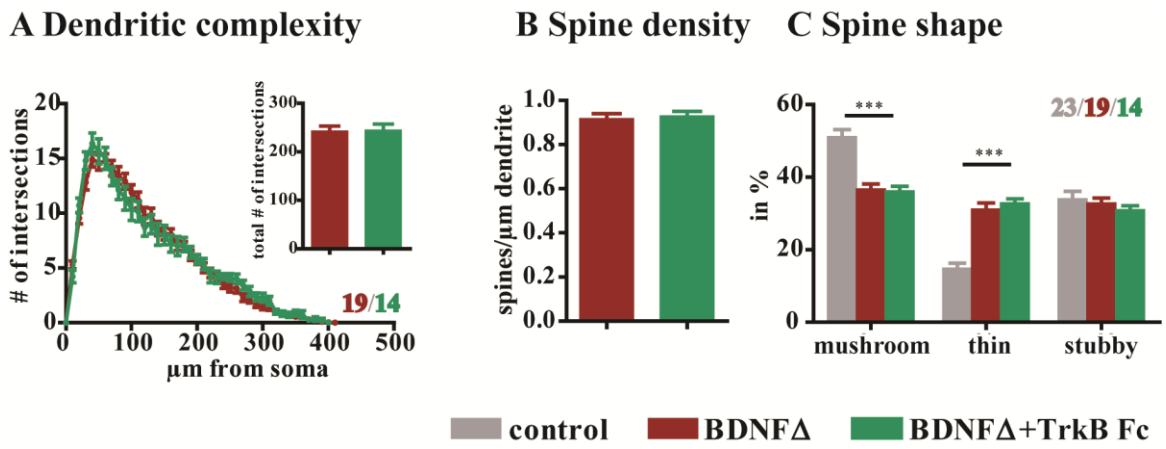

Figure 21 NT4 is not involved in maintain the neuronal morphology in BDNF depleted hippocampal primary neurons
A: Sholl analysis and total dendritic complexity (inserted graph) of hippocampal neurons remained unchanged after scavenging NT4 in already BDNF depleted cultures (BDNFΔ+TrkB Fc). **B:** The spine density was unaltered in BDNFΔ+TrkB Fc neurons compared to control neurons. **C:** The spine type distributions showed no additional effect in BDNFΔ+TrkB Fc neurons compared to BDNF depleted neurons. ****p*:0.001.

To examine whether NT4 might be involved in maintaining the dendrite morphology of hippocampal neurons in the absence of BDNF, primary neurons depleted of BDNF were treated with the BDNF and NT4 scavenger TrkB Fc. TrkB Fc (0.5 µg/ml) was applied to the medium of BDNF depleted primary neurons at 2, 4, 6, 12 and 17DIV. The neurons were visualized using the transfection with Lipofectamin2000 and fCherry and fixed at 21DIV. The dendritic complexity (Figure 21A) and the spine density (Figure 21B) remained unchanged in mature hippocampal neurons upon removal of NT4 in BDNF depleted primary cultures (BDNFΔ+TrkB Fc) compared to BDNF depleted neurons. The analysis of the spine type distribution in mature hippocampal neurons without BDNF and NT4 (BDNFΔ+TrkB Fc) revealed no additional effect when compared to only BDNF depleted neurons (Figure 21C).

Thus, the results demonstrate that binding of NT4 to the TrkB receptor is not able to rescue the lack of BDNF signaling in modulating dendritic structure, spine density and morphology in BDNF depleted hippocampal neurons.

3.7. Zinc is involved in maintaining the dendritic structure and spine density in hippocampal BDNF depleted neurons

The data presented so far suggest that the dendritic structure and spines density of excitatory hippocampal neurons in BDNF depleted primary cultures are maintained due to a BDNF-independent activation of the TrkB receptor. So far it is unclear how the TrkB receptor can be activated in the absence of its ligand BDNF. The results showed in 3.6. excluded a role of the neurotrophin 4 (NT4) in regulating the dendritic morphology and spine density in BDNF depleted neurons. Interestingly, a previous study reported a neurotrophin-independent activation of the TrkB receptor via the divalent cation Zinc, called TrkB transactivation (Huang et al., 2008;Huang and McNamara, 2010). Zinc can enter postsynaptic neurons and thereby phosphorylating the intracellular TrkB residues Y705/706 via the activation of the Src kinase (Huang and McNamara, 2010) thereby activating different signaling pathways downstream of the TrkB receptor. To test whether, in the absence of BDNF, the TrkB transactivation via Zinc may play a role in maintaining the dendritic structure of hippocampal neurons, BDNF depleted hippocampal cultures were treated at 1, 7 and 14DIV with CaEDTA as Zinc chelator (1.5 mM) (Frederickson et al., 2002;Huang et al., 2008). To determine the role of Zinc in regulating the neuronal morphology in culture with normal BDNF levels, control cultures were treated with CaEDTA. As the dendritic complexity and spine density of mature fCherry expressing control and BDNF depleted dissociated hippocampal neurons were indistinguishable (Figure 17A´´,B´´) the control and BDNF depleted hippocampal neurons treated with CaEDTA were compared to non-treated control hippocampal neurons. Notably, mature control neurons treated with CaEDTA (+CaEDTA) showed a significant decrease in total dendritic complexity and in the Sholl analysis compared to non-treated control hippocampal neurons (Figure 22A´´). However, the addition of CaEDTA to BDNF depleted cultures (BDNFΔ+CaEDTA) revealed an even stronger dendrite reduction than in control neurons (+CaEDTA) (Figure 22A´´). This effect was shown both in the Sholl analysis and total dendritic complexity where a significantly reduced dendritic complexity of BDNF depleted hippocampal neurons treated with

CaEDTA compared to control neurons treated with CaEDTA could be observed (Figure 22A´´).

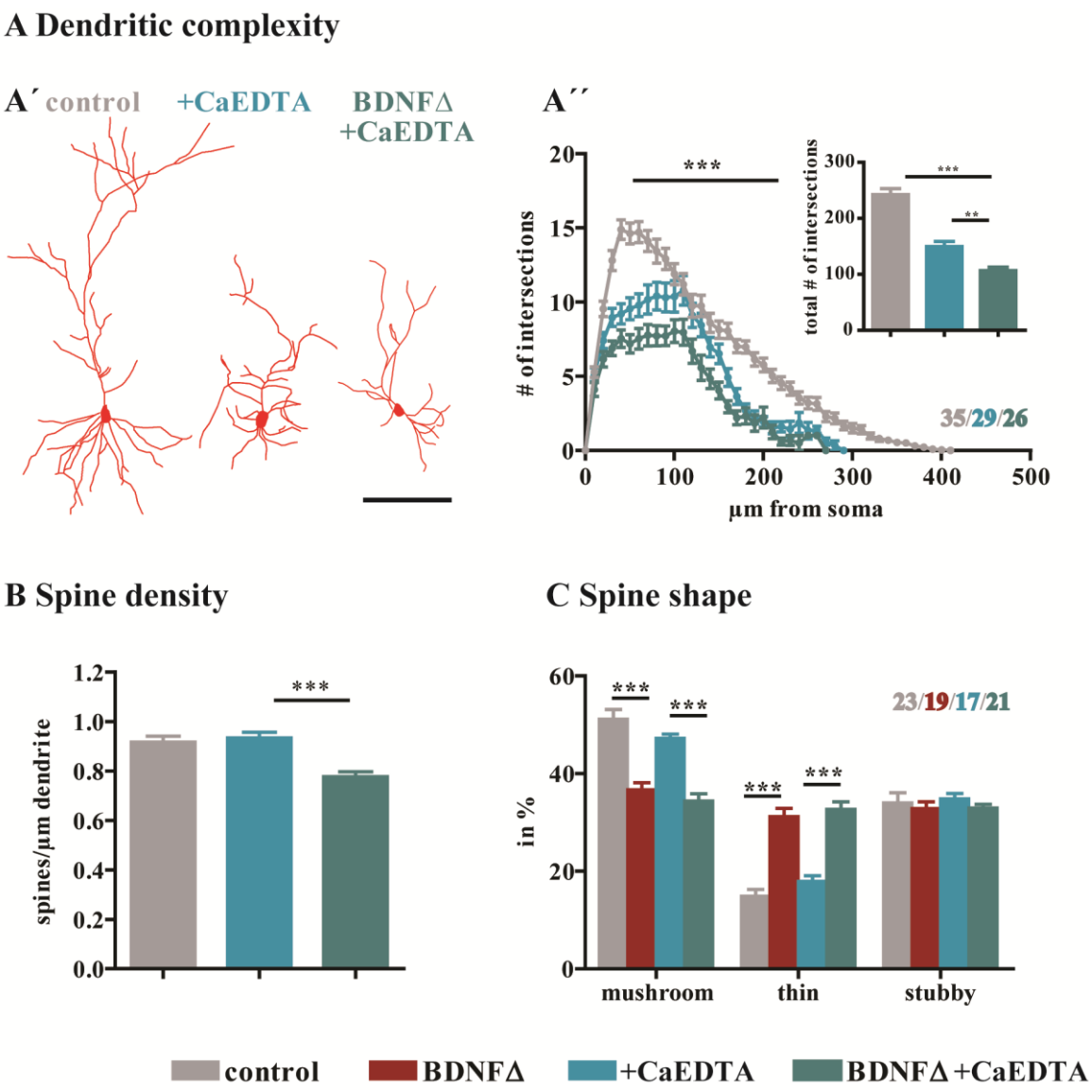

Figure 22 Zinc substitutes BDNF in regulating dendritic structure as well as spine density in BDNF depleted hippocampal neurons
Hippocampal primary neurons were treated with CaEDTA (1.5 mM) at 1, 7 and 14DIV. **A´:** Dendritic structure of hippocampal neurons after different treatments. **A´´:** Sholl analysis revealed a reduced dendritic complexity and total dendritic complexity (inserted graph) of mature control hippocampal neurons treated with CaEDTA (+CaEDTA) compared to nontreated neurons. The CaEDTA addition to BDNF depleted cells (BDNFΔ+CaEDTA) showed a significantly reduced dendritic complexity compared to control neurons treated with CaEDTA. **B:** the spine density remained unchanged after addition of CaEDTA to control neurons, whereas applying CaEDTA to BDNF depleted cells showed a significantly reduced spine density. **C**: Applying CaEDTA to control or BDNF depleted neurons exhibited no additional effect in spine type distribution. ***p*:0.01 and ****p*:0.001.

In a next step the spine density was determined of hippocampal neurons upon the above described treatments. While the spine density of control hippocampal neurons treated with CaEDTA exhibited no changes in comparison to control neurons, hippocampal

neurons from BDNF depleted cultures and treated with CaEDTA showed a significantly reduced spine density compared to either control or CaEDTA treated control neurons (Figure 22B). On the contrary to the effects of CaEDTA on dendritic complexity and spine density of hippocampal dissociated neurons, the analysis of the spine type distributions showed no additional effect upon CaEDTA treatment either in control or BDNF depleted hippocampal neurons (Figure 22C).

To investigate whether the Zinc mediated morphological changes of hippocampal dissociated neurons were restricted to the postnatal development, CaEDTA was added at a later time point (14DIV) to control and BDNF depleted hippocampal neurons. The neuronal morphology including dendritic complexity (Figure 23A), spine density (Figure 23B), and spine type distribution (Figure 23C), of mature hippocampal primary neurons revealed no difference upon CaEDTA treatment performed in control (+CaEDTA) or BDNF depleted cultures (BDNFΔ+CaEDTA) compared to non-treated control or BDNF depleted (BDNFΔ) hippocampal neurons.

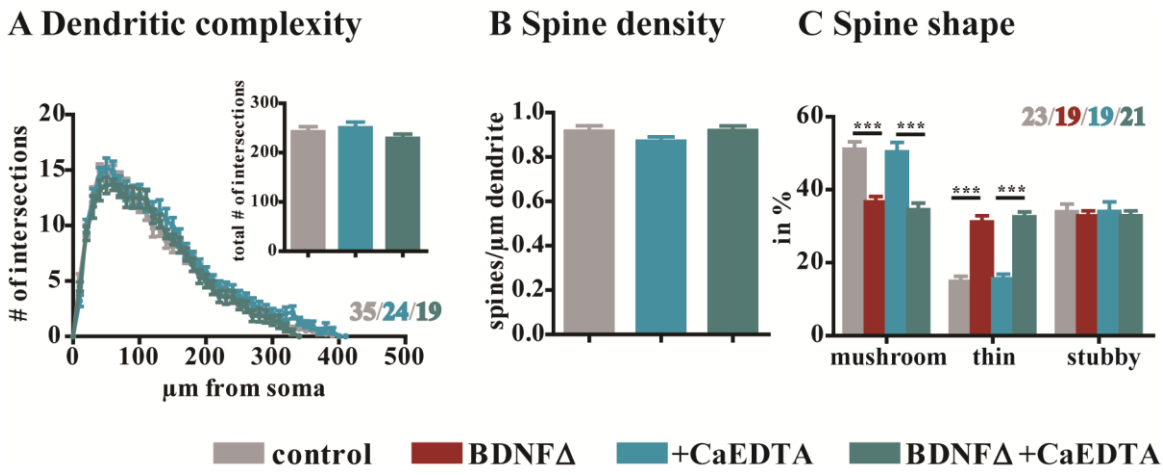

Figure 23 Neuronal morphology is unaltered after Zinc removal at later time point in BDNF depleted hippocampal neurons
CaEDTA (1.5 mM) was added at 14DIV to hippocampal primary neurons. The neuronal morphology including the dendritic complexity **(A)** and spine morphology (spine density **(B)** and spine shape **(C)**) was unaltered after CaEDTA application either to control or BDNF depleted neurons compared to non-treated control and BDNF depleted neurons. ***p:0.001.

Taken together, the above evidence leads to hypothesis that in the hippocampus in the absence of BDNF the TrkB receptor can be transactivated in a neurotrophin independent way *via* Zinc. This TrkB transactivation is able to maintain the dendritic structure and spine density of mature hippocampal neurons. However, in these neurons BDNF is

necessary for the mature spine architecture. In contrast, BDNF is not dispensable in regulating the postnatal growth of inhibitory neurons.

3.8. The actin binding Profilin2a modulates the neuronal morphology and is involved in activity dependent structural plasticity

The dynamic regulation of the actin cytoskeleton is essential for the ability of neurons to maintain their architecture as well as to undergo structural plasticity in response to changes in their environment. The actin binding protein profilin2a (PFN2a) is one of the major regulators of actin dynamics in neurons. However, how PFN2a contributes to maintaining the neuronal architecture such as dendrite morphology and spine number and shape is still unresolved. In this second part of my thesis, I investigated the involvement of the actin binding protein PFN2a in regulating the neuronal structure of mature hippocampal CA1 neurons. Moreover, I addressed the question of whether PFN2a is required for activity-dependent plasticity structural plasticity at dendritic spines.

Parts of the results described below have been recently published (Michaelsen et al., 2010)

3.8.1. Profilin2a is required for dendritic complexity and spine stability

A vector based PFN2a RNAi knockdown approach (shPFN2a) was used to examine the role of the actin binding protein profilin2a (PFN2a) in regulating neuronal morphology of hippocampal CA1 neurons. The shPFN2a is targeting the PFN2a mRNA and expressing farnesylated eGFP (feGFP) allowing us to visualize PFN2a-deficient neurons. As already shown the knockdown of PFN2a using shRNA takes at least 4 days (Michaelsen, 2009) and leads to a reduction of the PFN2a protein levels of 73.3 ± 2% (Michaelsen et al., 2010). A vector driving the expression of only feGFP or of shRNA against luciferase was used as controls. Organotypic hippocampal cultures were transfected with either feGFP, control shRNA or shPFN2a using a particle-mediated gene transfer at 7 days *in vitro* and imaged 1, 5, 7 and 9 days post-transfection (dpt) to document the changes in their dendritic morphology upon PFN2a knockdown and compare it with the one of control neurons. To determine whether the dendritic length is impaired after knockdown of PFN2a, the total dendritic length of neurons at 1dpt was set as 100%. All following time points were calculated in proportion to 1dpt. While dendrites in control cells transfected

with feGFP exhibited no alteration (Figure 24B), five days after transfection (5dpt) with shPFN2a, CA1 neurons showed retraction and pruning of pre-existing dendrites (Figure 24A). The control neurons, either transfected with feGFP or shRNA against Luciferase showed no reduction in dendritic length after 9dpt, whereas the quantification of changes in total dendritic length for the apical dendrite of shPFN2a CA1 neurons compared to control neurons revealed a reduction by about 30% at 5dpt and even more pronounced at 7 and 9dpt respectively (Figure 24C).

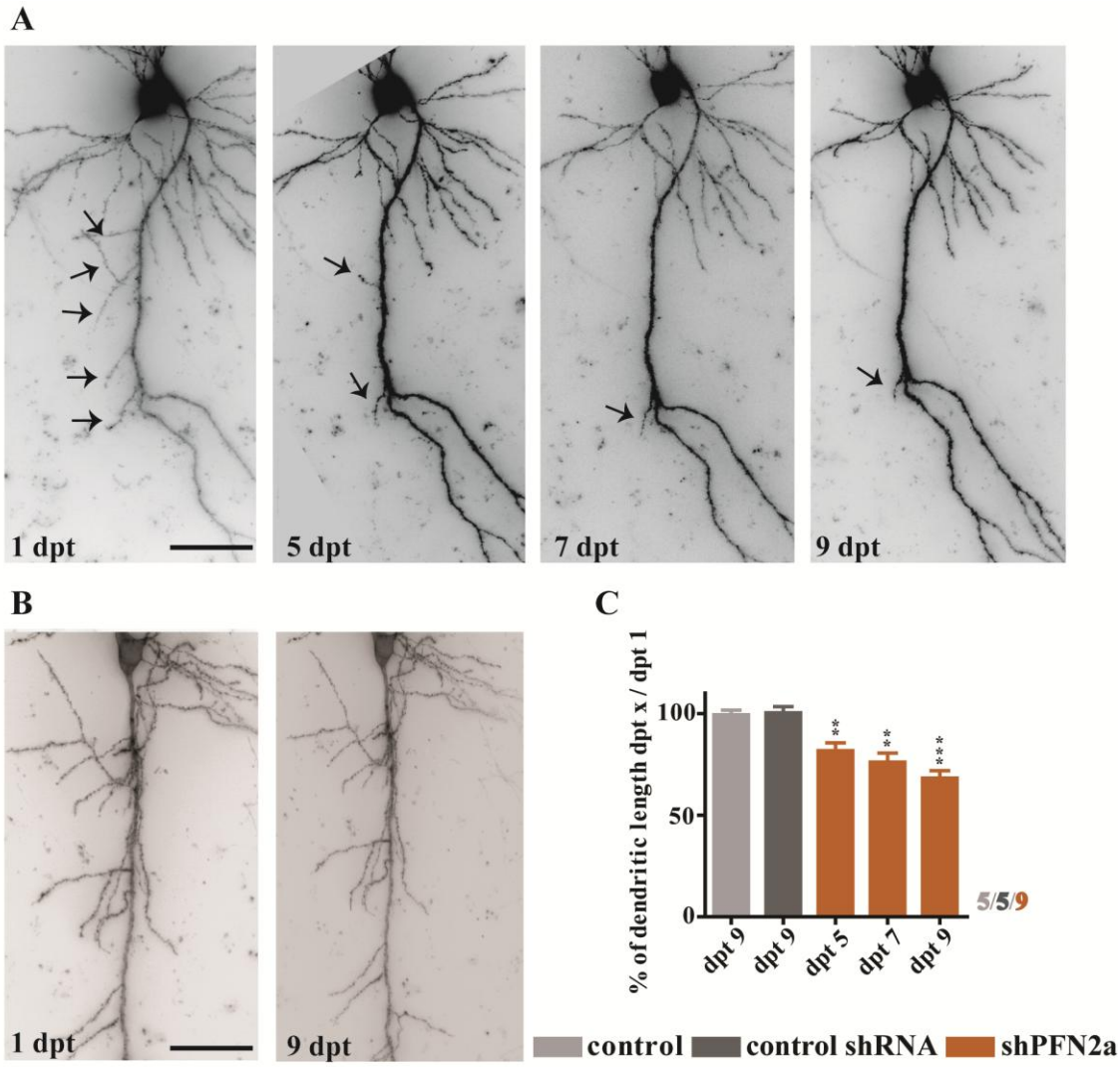

Figure 24 Dendritic retraction in PFN2a-deficient cells
A: shPFN2a-expressing CA1 hippocampal neuron in organotypic slice culture imaged at 1, 5, 7 and 9 days post transfection (dpt). Pruning of dendrites at the apical part started at 5dpt indicated with arrowheads. **B:** feGFP expressing cell remained stable within the imaged time period. **C:** quantification of dendritic length revealed stable dendritic length of control (feGFP) and control shRNA after 9dpt, whereas the dendritic length of shPFN2a-expressing cells showed a significant reduction after 5dpt and even more after 7 and 9 dpt. ***p*:0.01 and ****p*:0.001.

These data support the results of a PFN2a knockdown performed in 7DIV hippocampal neurons which were fixed and analyzed at 7dpt. Detailed morphological analysis, including the Sholl analysis and spine density counting were performed in these neurons (Michaelsen, 2009;Michaelsen et al., 2010). The Sholl analysis revealed an impaired neuronal morphology upon PFN2a knockdown in fixed CA1 neurons. The complexity of the basal and proximal apical dendritic compartments was significantly reduced when compared to control neurons. Moreover, a significantly decreased spine density was observed both in the basal and mid-apical dendrites of shPFN2a-expressing CA1 hippocampal neurons (Michaelsen et al., 2010). The lower spine density observed in the dendrites of shPFN2a-expressing could be explained by two different processes: PFN2a might be involved either in regulating the formation of new dendritic spines or in controlling their maintenance. To distinguish between these two possibilities, time lapse imaging was performed for dendritic stretches of control and PFN2a-deficient CA1 neurons. As they are known to receive inputs from different origins, the basal and mid apical (proximal and distal) dendrites, of CA1 hippocampal neurons were analyzed separately. To document the spine density over the imaging time period (1, 5, 7, and 9dpt) one stretch for each of the different dendritic compartments was chosen repetitively imaged at the different time points. As shown above (Figure 24A), also this imaging series showed a retraction and pruning of dendrites in the mid apical (proximal and distal) compartment of PFN2a-deficient CA1 neurons. In contrast to that, no change or a slight increase in dendritic length could be observed in control neurons for the proximal and distal mid apical compartment respectively (Figure 25A). Moreover, while the dendrites in the basal compartment of control neurons increased in length, those in PFN2a-deficient CA1 neurons failed to grow during the imaging period (Figure 25A). Interestingly, the spine density of control neurons in the basal and distal mid apical dendrites was increasing throughout the imaging time. On the contrary, in shPFN2a-expressing cells no progressive increase in spine density could be detected (Figure 25B). No alteration between the control and PFN2a-deficient cell could be observed in the proximal apical compartment Taken together these results suggest that PFN2a indeed influences dendritic spine density by promoting the formation of new spines rather than modulating their maintenance.

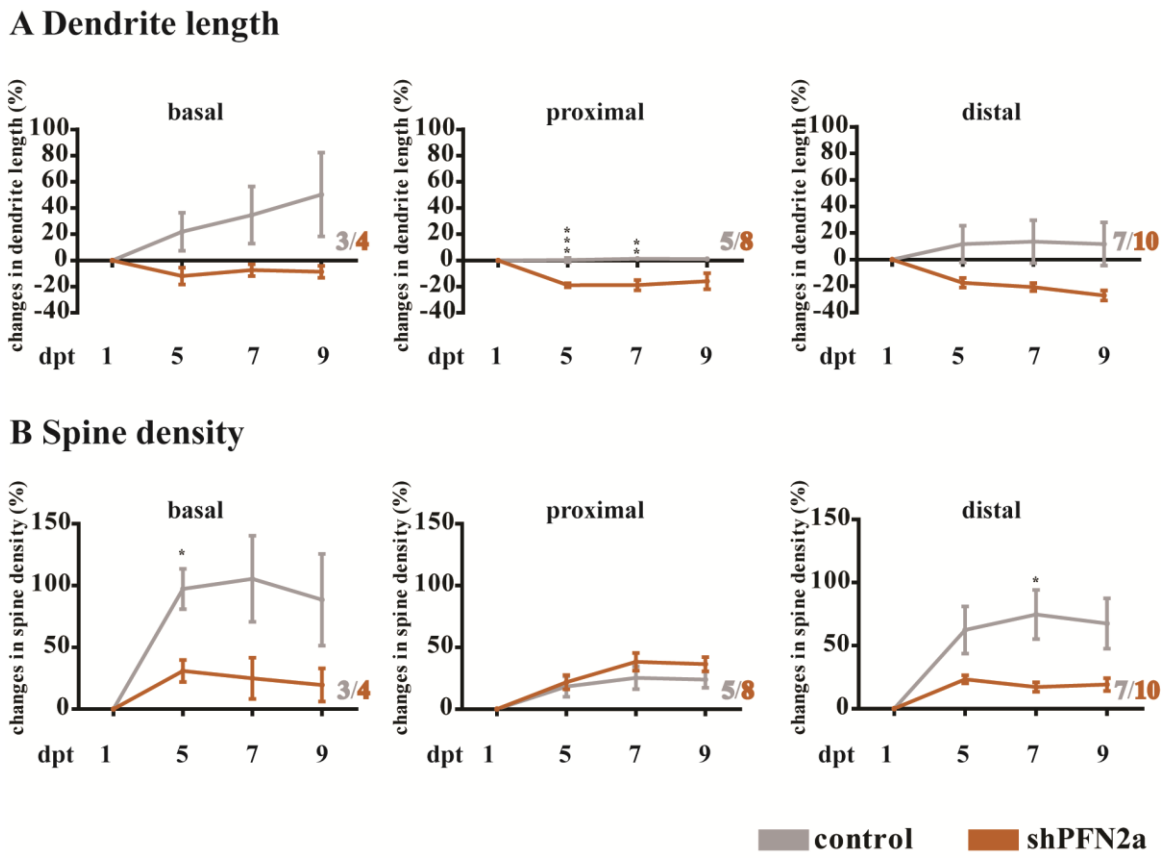

Figure 25 PFN2a is involved in dendrite stability as well as in spine density
A: The observed dendritic length at the indicating time points was varying between the subcompartments of control CA1 neurons. Whereas the dendrites in the basal part gained in length during the imaging time period, dendrites in the apical part remained unchanged. However, dendrites of PFN2a-deficent cells lost dendritic length during the imaging period**. B:** The increased spine density in control neurons in the basal and distal apical part could not be detected in shPFN2a-expressing cells during the imaging period. In the proximal apical dendritic part spine density of feGFP and shPFN2a transfected cells was not significantly different. **p*:0.05; ***p*:0.01; ****p*:0.001.

Besides spine density, changes in spine shape and dynamics are dependent on actin dynamics and are supposed to reflect changes in strength of synaptic transmission (von Bohlen und, 2009). Therefore, in the next set of experiments possible changes in spine stability were analyzed in shPFN2a-expressing CA1 hippocampal neurons. To this aim the spine morphology and motility over time were compared between PFN2a-deficient and control CA1 neurons. First the spine type distribution upon PFN2a knockdown was analyzed in neurons fixed at 9dpt. Depending on their shape, dendritic spines can be subdivided into different categories (mushroom, thin and stubby) based on the relative sizes of the spine head and neck and in relation to the spine length (Harris et al., 1992). Basal and apical dendritic compartments were analyzed separately for feGFP and shPFN2a transfected neurons. PFN2a deficient neurons showed significant differences in spine type distribution in both, basal and apical dendrites when compared to control

neurons (Figure 26A). Interestingly, the alterations observed are opposite in apical and basal compartments. Basal dendrites of shPFN2a-expressing cells revealed a significant reduction of mushroom spines and an increase in stubby spines. On the contrary, in the apical dendrites the stubby spine were significantly increased and the proportion of mushroom spine reduced compared to control neurons (Figure 26A). To further investigate whether the spine dynamics might be affected upon PFN2a knockdown, time lapse imaging of defined dendritic branches was performed at 9dpt. A dendritic segment was imaged every 5 min for 20 min in total and spine length as well as spine head diameter were measured of all dendritic spines at the different time points. The spine dynamics were quantified as changes in spine length or spine head diameter per 5 min or using the motility index showing changes in length over the whole imaging session (20 min). As no significant difference in spine dynamics could be observed between the apical and basal compartment of control and shPFN2a-expressing neurons, the data were pooled. Control neurons showed a 5% change and a motility index around 0.1 µm in their spine length and spine head during the imaged period of 20 min. In contrast to control neurons, PFN2a deficient CA1 hippocampal neurons showed a highly significant increase in their spine changes (spine length: 10%; spine head 17%) and motility (spine length: 0.58 µm; spine head 0.56 µm) (Figure 26B´, B´´). Interestingly, the increased motility in shPFN2a-expressing neurons could be partially rescued by overexpressing of RNAi resistant PFN2a (shPFN2a/PFN2a) (Figure 26B´, B´´).

Taken together, these results support our recently published results indicating a crucial function for PFN2a in controlling the fine tuning of dendritic structure as well as spine density (Michaelsen et al., 2010). Additionally, the time lapse imaging revealed that PFN2a is as well important in maintaining the dendritic spine stability.

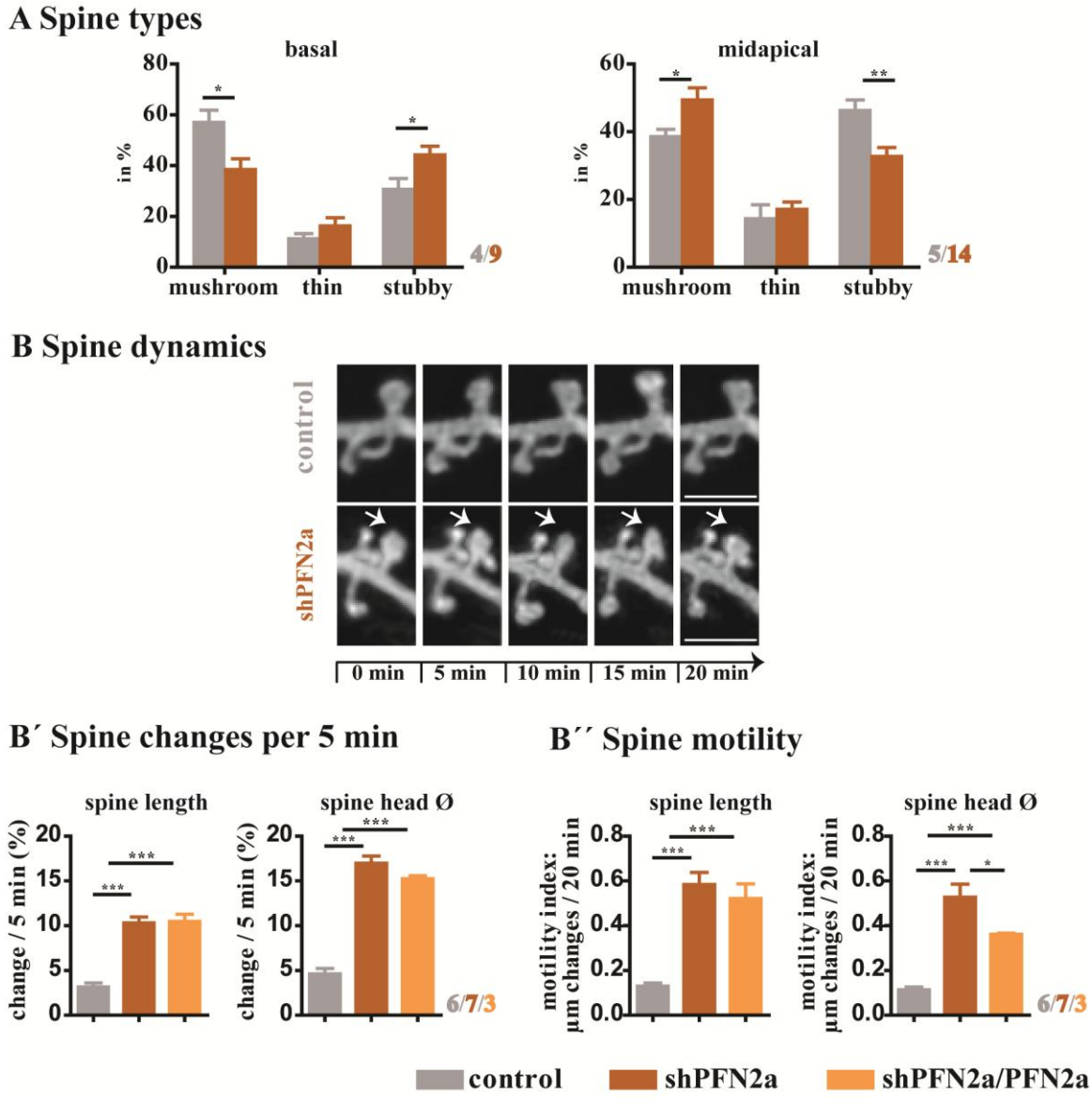

Figure 26 Spine type distribution and spine dynamics are significantly altered in PFN2a-deficient CA1 neurons
A: In basal dendrites of CA1 hippocampal neurons, mushroom spine types were significantly reduced in PFN2a-deficient neurons, while stubby spine increased. Interestingly, the opposite spine type distribution could be observed in the mid apical part of CA1 shPFN2a-expressing cells compared to control neurons. **B:** Spines images of control (feGFP) and shPFN2a transfected neurons at the indicated time points. **B´:** Spine changes per 5 min revealed a significant increase in shPFN2a-expressing cells, as well as neurons with an overexpression of a RNAi resistant PFN2a in PFN2a-deficient cells compared to control neurons. **B´´:** The increased spine motility in the spine head diameter could be rescued by overexpressing the RNAi resistant PFN2a. **p*:0.05; ***p*:0.01; ****p*:0.001.

3.8.2. Profilin2a is involved in activity dependent structural plasticity

Dendritic spines can undergo bidirectional activity-dependent morphological changes (Nagerl et al., 2004). High frequency stimulation led to the growth of spines, while after a low frequency stimulation the pruning of existing spines could be observed (Nagerl et al.,

2004). Recent data suggested an involvement of the dynamic actin cytoskeleton in mediating these morphological changes of spines (Matus et al., 2000). Indeed not only the major cytoskeleton protein, filamentous F-actin is concentrated in dendritic spines (Matus et al., 1982), but also the LTP induction results in a shift in the G-actin/F-actin ratio toward F-actin in spines (Fukazawa et al., 2003). Interestingly, it was shown before that two of the isoforms of the actin-binding proteins profilin1 and 2a (PFN1 and PFN2a) are targeted to dendritic spines in an activity-dependent manner (Ackermann and Matus, 2003;Neuhoff et al., 2005). To investigate whether PFN2a plays a role in mediating structural activity-dependent plasticity upon induction of long-term potentiation, a well-described chemical protocol (10 mM Glycin) was used to increase global activity and induce NMDA receptor-dependent long-term potentiation in the hippocampal slice culture (Shahi et al., 1993) comparable to LTP induction *via* the common theta burst protocol (Fortin et al., 2010).

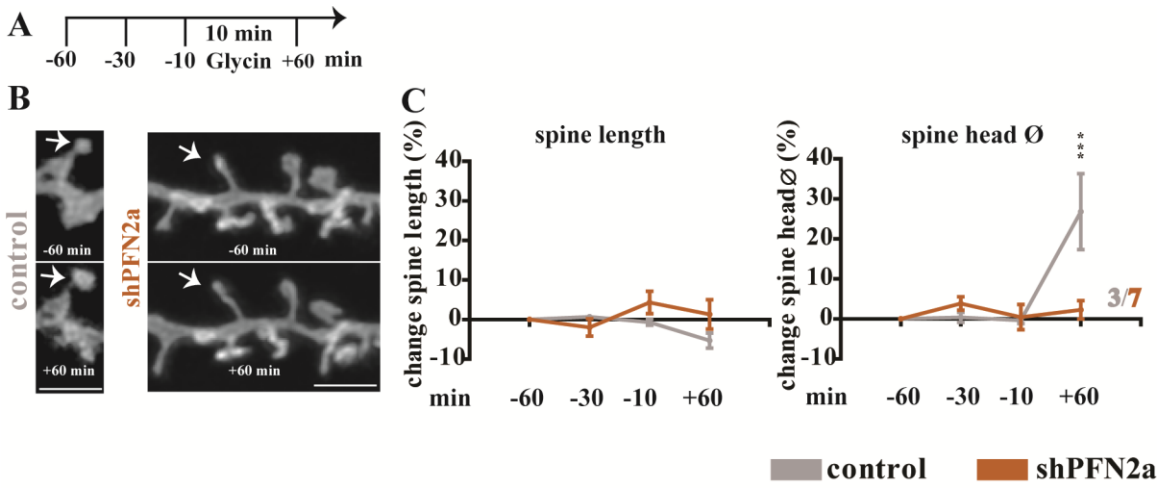

Figure 27 PFN2a is crucial for structural plasticity
A: Imaging time course for chemical long term potentiation (cLTP) induction using 10 mM Glycin. **B:** Spines of control (feGFP) or shPFN2a transfected CA1 neurons 60 min before and 60 min stimulation. **C:** Spine length was not changed upon cLTP stimulation in feGFP and shPFN2a transfected cells. While the spine head diameter of control cells showed an increase after stimulation, in PFN2a-deficient neurons this increase could not be detected. ****p*:0.001.

As expected (Fortin et al., 2010), 60 min after chemical LTP induction, feGFP expressing neurons showed a significant increase of 25% in spine head diameter (Figure 27C). In contrast to that no change in spine head diameter could be observed 60 min after stimulation PFN2a-deficient neurons (Figure 27C).The spine length was not altered both in control and shPFN2a-expressing neurons after stimulation (Figure 27C).

Taken together, the actin binding protein PFN2a is crucial for the neuronal morphology of CA1 hippocampal neurons including the dendrite and spine stabilization. It seems that PFN2a is important for the maintenance of dendritic structure, while PFN2a mediates the spine density formation. Moreover, PFN2a modulates the activity-dependent structural plasticity of dendritic spines.

4. Discussion

The development and maintenance of the neuronal structure is crucial for ensuring a correct brain function. The neuronal architecture is tightly regulated *via* extracellular signals able to stabilize or change the neuronal structure. The current study revealed new insights into the regulation of neuronal morphology in the postnatal and adult brain.

First, I could show that the neurotrophin BDNF regulates the neuronal architecture in a cell type-specific way. While BDNF is required for postnatal growth of inhibitory neurons in the cortex and hippocampus, it is not required for postnatal growth of excitatory neurons in the same brain areas. Moreover, I addressed the molecular mechanism mediating the differential effect of BDNF in regulating neuronal morphology of inhibitory and excitatory neurons. Notably, despite the loss of its main ligand, the TrkB receptor was still phosphorylated in excitatory neurons, but not in inhibitory neurons. Interestingly, I could show that the neurotrophin 4 (NT4) is not able to compensate the loss of BDNF in hippocampal neurons. Remarkably, my data show that Zinc is involved in regulating the dendritic structure and spine density of hippocampal neurons through a neurotrophin-independent transactivation of the TrkB receptor.

Furthermore, I could show a specific role for the actin binding protein, profilin 2a (PFN2a) in mediating neuronal morphology. In PFN2a-deficient neurons the retraction or pruning of pre-existing dendrites could be observed and the spine density formation was impaired. Moreover, the spine motility was significantly increased in PFN2a-deficient neurons. Additionally, a crucial role for PFN2a could be observed in the activity-dependent structural plasticity, as spine heads failed to grow upon induction of chemical long-term potentiation in cells down regulating PFN2a.

4.1. The effects of a global BDNF deletion are surprisingly area-specific

The role of BDNF in regulating neuronal morphology in the postnatal brain has been so far very difficult to assess as *bdnf -/-* mouse mutants die too early for the role of BDNF to be assessed after its marked postnatal increase (Maisonpierre et al., 1990; Zafra et al., 1990; Castren et al., 1992). Different conditional *bdnf* knockout mice have been generated to circumvent this problem using the cre/loxP system to delete BDNF in a temporal- or area-specific manner. (Rios et al., 2001;Gorski et al., 2003;Baquet et al.,

2004;He et al., 2004;Chan et al., 2006;Chan et al., 2008;Monteggia et al., 2007;Unger et al., 2007). However, none of these conditional *bdnf* knockout mice achieve a global deletion and the anterograde axonal transport of BDNF from different areas complicates the interpretation of the results (Altar et al., 1997). A newly generated BDNF conditional knockout *(cbdnf ko)* mouse where *bdnf* excision mediated by a cre recombinase inserted in the *tau* locus, a gene expressed in postmitotic neurons (Tucker et al., 2001) results in the almost complete disappearance of BDNF throughout the brain and spinal cord (Rauskolb et al., 2010) and avoids thereby the anterograde transport of BDNF. This *cbdnf ko* mice survive for several month. Surprisingly, the effects observed upon this global BDNF deletion indicate an area-specific requirement of BDNF. On the one hand, the volume of the hippocampus in *cbdnf ko* mice is unaltered and only a mild alteration in the dendritic architecture of the pyramidal neurons in this area could be observed. On the other hand, the volume of the striatum of *cbdnf ko* mice is significantly reduced due to the significant simplification of the dendritic structure of medium spine neurons (MSNs), the predominant neuronal population in the striatum (Rauskolb et al., 2010). Remarkably, the specificity of the effect of BDNF observed *in vivo* in *cbdnf ko* mice could be reproduced upon BDNF depletion *in vitro*, where primary culture of $bdnf^{lox/lox}$; tau^{wt} mice were infected with a virus carrying a vector expressing the cre recombinase. In this context, the MSNs were reduced in their dendritic complexity, while hippocampal neurons revealed normal dendritic complexity and spine density, but a significantly altered spine type distribution in BDNF depleted neurons. These results leave open two different hypotheses. The effect of BDNF could be an area- or cell type specific-effect. Indeed, in our previous analysis we compared neurons in the striatum with neurons of the hippocampus as well as inhibitory neurons (MSNs) with excitatory neurons (pyramidal cells). The area-specific effect of BDNF would imply that the function of BDNF is different in the hippocampus and striatum, while the cell type-specific effect would suggest a different regulation of BDNF in inhibitory and excitatory neurons.

4.2. BDNF differentially regulates the dendritic architecture of excitatory and inhibitory neurons throughout the brain

In order to distinguish between possible area- or cell type-specific effects of BDNF, the dendritic structure of inhibitory and excitatory neurons within the same brain areas were investigated. First, I analyzed the neuronal morphology of another excitatory neuronal

population in the cortex to examine if besides excitatory neurons in the hippocampus, the neurons in the cortex show the same morphological effect upon loss of BDNF. Interestingly, the volume of the cortex in *cbdnf ko* mice is reduced by about 20% (Rauskolb, 2008), while the volume of the hippocampus is unaltered (Rauskolb et al., 2010). The excitatory neurons in the cortical layer II/III and layer V of *cbdnf ko* mice showed a mild effect in their dendritic structure. This is consistent with the results observed before in CA1 pyramidal neurons of these mice. A previous study showed in 5 weeks old *Emx-bdnfko* mice a reduced complexity for the basal compartment of pyramidal neurons in layer II/III of the visual cortex which could not be seen in our analysis (Gorski et al., 2003). This difference could be explained by the different experimental conditions used in the two studies. Different mice were used in the current study and in the study of Gorski and colleagues (2003). While the *cbdnf ko* mice used in this work show a 95% reduction of BDNF (Rauskolb et al., 2010), the BDNF deletion in *Emx-bdnfko* mice is restricted to the forebrain. This global depletion of BDNF in our mouse could activate compensatory mechanism, while the left BDNF in the other brain areas in the forebrain restricted mouse could suppress other mechanisms to regulate the dendritic structure, like the other TrkB ligand neurotrophin 4 (NT4) or a neurotrophin-independent mechanism of TrkB activation. Moreover, they investigated neurons in the visual cortex, while neurons analyzed in this work were chosen from the entire cortex and were not separated between different cortical areas. Thus, it could be that no neurons from the visual cortex were included in our analysis or that the effect of BDNF is restricted to the basal compartment of layer II/III pyramidal neurons in the visual cortex and pooled data from the entire cortex neutralized this effect. Furthermore Gorski and colleagues saw a significant increase of NT3 in the forebrain after BDNF deletion in their mutant mouse. Interestingly a previous study found an opposite role of BDNF and NT3 in regulating cortical dendritic growth (McAllister et al., 1997), where NT3 inhibits BDNF dependent dendritic growth of layer IV pyramidal neurons. Possibly, the increased NT3 in *Emx-bdnfko* mice could cause a negative effect in neuronal morphology of pyramidal neurons in layer II/III. In the *cbdnf ko* mice used in this study the NT3 levels are not changed compared to control mice (Rauskolb, 2008).

To address the possibility of a cell type-specific effect possible changes in the dendritic architecture of different inhibitory neurons upon BDNF deletion were analyzed in the cortex and hippocampus where excitatory neurons revealed only minimal alterations in their structure. In those regions, the inhibitory neurons demonstrated a significant loss in

their dendritic complexity upon BDNF depletion both *in vivo* and *in vitro*. This is in line with previous studies, where inhibitory neurons of the cortex as well as hippocampus are shown to be dependent on BDNF in their development (Vicario-Abejon et al., 1998;Kohara et al., 2003). In this study, an antibody staining against three calcium binding proteins, parvalbumin, calbindin and calretinin was used to distinguish the different subpopulations of GABAergic neurons to investigate whether the BDNF effect is a general effect on inhibitory neurons or a specific effect on a subpopulation. A previous study showed that different subpopulations of inhibitory neurons are differently regulated by BDNF (Marty et al., 1996). In positive interneurons these proteins are distributed throughout the cell soma and dendrites making it feasible to use the immunhistochemical staining to investigate their dendritic architecture. Due to the weak signal in the distal and thin dendritic branches it is possible that the absolute dendritic complexity was here underestimated. However, the relative changes in dendritic complexity between control and BDNF depleted neurons revealed a strong decrease in the latter and exhibited that all three different subpopulations of inhibitory neurons are dependent on BDNF in their postnatal growth.

Taken together, inhibitory neurons in the striatum (Rauskolb et al., 2010), as well as in the hippocampus and cortex revealed a significantly reduced dendritic complexity upon BDNF loss, whereas excitatory neurons of the hippocampus and cortex showed a mild phenotype under the same conditions. These results suggest that BDNF regulates the dendritic morphology in a cell type-specific manner. In support to this conclusion is the observation that in *cbdnf ko* mice the volume of the cortex is significantly smaller compared to the one of control mice (Rauskolb, 2008). This volume reduction could indeed be explained by the significant reduction observed in the dendritic complexity of inhibitory neurons, as the cortex consists of 20% inhibitory neurons. Moreover, the normal volume of the hippocampus could be due to the fact that the hippocampus consists of only 5% inhibitory neurons, while the strong volume reduction in the striatum of *cbdnf ko* mice is caused by the significantly reduced dendritic complexity of inhibitory MSNs, which are the main population in the striatum. Furthermore, the analysis of spine morphology in *cbdnf ko* mice supported the role of BDNF to be cell type-specific. Indeed, while spine density in inhibitory MSNs is significantly reduced (Rauskolb et al., 2010) hippocampal and cortical excitatory neurons revealed no alteration in their spine density in *cbdnf ko* mice. Only spine type distribution showed a similar significant alteration for pyramidal neurons in both brain areas, hippocampus (Rauskolb et al., 2010) and cortex. It

is noteworthy that the shifted spine type distribution could be rescued by applying BDNF to BDNF depleted neurons indicating that the changes in spine type distribution are due to the loss of BDNF. Interestingly, applying BDNF to control hippocampal neurons resulted in dendritic elongation, but induced no changes in spine density and spine type distributions. It was reported before that BDNF treatment induces the elongation of neurites of developing hippocampal neurons (Ji et al., 2010;Thieme, 2011), supporting the results of the current study. On the other hand, previous studies could show that the spine density (Ji et al., 2005) and the spine head diameter (Ji et al., 2010) are increased after BDNF application. On the contrary, work from our laboratory showed no effects on spine morphology upon exogenous BDNF application to hippocampal neurons (Kellner, 2010). This discrepancy between Ji and colleagues and the current work could be explained by different cell culture condition, as already the initial cell density which plays a crucial role for forming synaptic contacts in primary neurons is lower in the study of Ji and colleagues than in the current study. Thus, neurons in a lower cell density with less synaptic contacts could respond to the application of BDNF.

Taken together, these data indicate that the effect of BDNF in regulating the neuronal morphology during postnatal development is cell-type specific. BDNF is required for the postnatal growth of GABAergic inhibitory neurons, but not for excitatory neurons.

4.3. Molecular mechanisms compensating for the absence of BDNF in specifically regulating the architecture of excitatory neurons

In the attempt to understand the molecular mechanisms mediating the different effects of BDNF on the dendritic structure of inhibitory *versus* excitatory neurons I investigated whether differences in the activation of the BDNF receptor TrkB in the two neuronal populations could be observed. Due to its role as positive regulator of neuronal morphology (Lu et al., 2005), it is likely that the BDNF receptor TrkB might still be able to modulate the dendritic structure of excitatory neurons even in the absence of BDNF. Remarkably, we could show that despite the complete BDNF depletion, the TrkB receptor was still phosphorylated in excitatory hippocampal neurons. Specifically, the phosphorylation occurred at the site Y705/706, which is located within the autoregulatory loop. In contrast, inhibitory MSNs showed no TrkB phosphorylation after BDNF depletion and therefore no activation of the TrkB receptor. Thus, in excitatory neurons TrkB is activated independently of BDNF. The neurotrophin 4 (NT4) can also bind to

TrkB, while a neurotrophin-independent mechanism *via* Zinc is able to activate the TrkB receptor as well (Huang and McNamara, 2010). In the next sets of experiment I addressed the two possibilities of NT4 and Zinc being able to mediate the activation of TrkB in the absence of BDNF and thereby promoting the neuronal stability of excitatory neurons.

4.3.1. Neurotrophin 4 (NT4) does not compensate the absence of BDNF in regulating the dendritic structure of excitatory neurons

NT4 can bind to the TrkB receptor, thereby inducing positive signaling transduction (Chao, 2003). However, removing NT4 in BDNF depleted primary hippocampal cultures induced no alteration in the dendritic structure and spine density when compared to only BDNF depleted neurons. Moreover no additional effect could be observed in spine types distributions. Thus, NT4 is not involved in regulating the neuronal architecture in hippocampal neurons upon BDNF depletion. In contrast to our data, a recent study showed that NT4 can substitute BDNF functions *in vivo* (Fan et al., 2000). NT4 supports the survival of sensory neurons and promotes functional synapse formation in cultured hippocampal neurons in the absence of BDNF. In their study NT4 is expressed under the BDNF promoter resulting in the same expression pattern and activity-dependent release of BDNF. However, it was reported before that under physiological conditions BDNF and NT4 exhibit different expression pattern throughout the brain (Timmusk et al., 1993). On this account the location, timing and amount of secreted NT4 and BDNF seem to be crucial for their different functions in the brain *in vivo*. Moreover, Minichiello and colleagues (1998) pointed to different TrkB pathways upon either BDNF or NT4 binding. NT4-dependent effects are predominantly modulated through the MAPK pathway, while BDNF effects could be obtained through all three TrkB signaling pathways.

Thus, the results demonstrate that NT4 binding to the TrkB receptor is not the mechanism substituting the lack of BDNF in regulating dendritic structure and spine density in BDNF depleted hippocampal neurons. Hence, the data indicate a neurotrophin-independent TrkB phosphorylation in hippocampal neurons.

4.3.2. Zinc can substitute BDNF to regulate dendritic structure and spine density in hippocampal neurons

Previous studies reported that TrkB can be phosphorylated and transactivated in a neurotrophin-independent way *via* the divalent cation Zinc (Huang et al., 2008;Huang and McNamara, 2010). While Zinc containing neurons are glutamatergic, not all glutamatergic neurons contain Zinc. Zinc is stored together with the excitatory transmitter glutamate in neurons of the neocortex, amygdala and is especially concentrated in the mossy fiber boutons in the hippocampus (Frederickson et al., 2000). Upon physiological stimulation, Zinc is released together with glutamate and enters the postsynaptic neurons through voltage-gated calcium channels (VGCCs) and NMDA receptors (Huang et al., 2008). Zinc can activate the Src family kinases (SFK) (Src, Fyn and Yes) and thereby promotes the phosphorylation of the tyrosine residues Y705/Y706 and Y816 (PLCγ pathway) on the TrkB receptor (Huang and McNamara, 2010). Interestingly, removing Zinc in control hippocampal neurons already reduced their dendritic complexity compared to non-treated neurons. BDNF and Zinc depleted hippocampal neurons showed an even higher reduction of dendritic complexity compared to neurons without Zinc but with normal BDNF levels. The spine density was not different after Zinc removing in control neurons, whereas BDNF and Zinc depleted hippocampal neurons demonstrated a significantly reduced spine density. On the other hand, the spine type distribution showed no additional effect after Zinc removing in control or BDNF depleted neurons. The data suggest that Zinc can compensate the absence of BDNF in regulating both, dendritic structure and spine density in excitatory hippocampal neurons, but it is not able to substitute the BDNF effect on spine type distribution. Regarding the regulation of the dendritic structure, it seems that Zinc can compensate the lack of BDNF, while BDNF needs Zinc for its complete function. The analysis of the spine density revealed that *in vitro* BDNF and Zinc can substitute each other in controlling spine number. The action of Zinc is restricted to the neuronal development, as removing Zinc at a later time point (14DIV) induced no changes in neuronal morphology. A different amount of Zinc during development and adulthood and therefore a more sensitive responsiveness in neuronal structure upon removing Zinc during development could be one explanation why the action of Zinc is restricted to neuronal development. On the other hand it was recently shown that Zinc is involved in regulating neuritogenesis both during development and adulthood (Levenson and Morris, 2011). One possible explanation for these differences is that, Zinc may be important for the neuronal development, but not for the maintenance of

the mature structural. Taken together, it can be concluded that Zinc is important to regulate the general neuronal structure but not for the fine tuning of spines in the more advanced stages of development. As neuronal structure is correlated to the long term potentiation (LTP) (Engert and Bonhoeffer, 1999), the following question arises: Why does Zinc regulate the dendritic structure and the spine density and not the long term potentiation (LTP) in the hippocampus after BDNF loss? It was shown that the LTP induction at the Shaffer collaterals of the hippocampus is impaired in BDNF knockout mice (Korte et al., 1995;Patterson et al., 1996). On the other hand, a previous study reported that exogenous zinc potentiates the efficacy of the hippocampal mossy fiber-CA3 pyramid synapse by a TrkB-requiring mechanism. The LTP is impaired by removing Zinc (Huang et al., 2008). Indeed, Zinc is important to mediate the LTP in the hippocampus, possibly it is involved predominantly at the mossy fiber-CA3 long term potentiation, as its highest concentration is found at the mossy fiber boutons in the hippocampus. Furthermore, the effect of Zinc on dendritic complexity was analyzed *in vitro*, while the long term potentiation experiments were done *in vivo*. Finally, the transactivation via Zinc and the activation via BDNF triggered different patterns of phosphorylation of the TrkB receptor. Y705/706 (residue in the autoregulatory loop) and Y816 (PLCγ pathway) are phosphorylated *via* Zinc and BDNF, while the tyrosine residue Y515 (MAPK pathway) is phosphorylated only upon BDNF binding. However, it is published that the phosphorylation of Y515 is not required for LTP (Korte et al., 2000). Thus, the distinct phosphorylation pattern does not explain why Zinc is not involved in regulating the induction of LTP at the Shaffer collaterals, as Zinc preferentially phosphorylates the PLCγ pathway.

Taken together, the data indicate that during postnatal development BDNF regulates the dendritic architecture of neurons in a cell type-specific way. While, BDNF is required for postnatal growth of inhibitory neurons in the striatum, it is dispensable for the growth of excitatory neurons in the cortex and hippocampus. Furthermore, this study shows that in the absence of BDNF the dendritic structure and spine density in excitatory neurons is regulated *via* a Zinc-mediated transactivation of the TrkB receptor. In excitatory neurons BDNF is required to modulate spine shape.

4.4. Profilin2a is crucial for neuronal morphology

Two different isoforms of the actin binding protein profilin are expressed in the mammalian brain. While profilin2a (PFN2a) and profilin1 (PFN1) are expressed together throughout the brain (Witke et al., 1998), the ratio of PFN2a to PFN1 is higher in brain areas known for their high levels of activity-dependent plasticity, like the hippocampus and the cortex (Lambrechts et al., 2000). The main function of profilin is to regulate the actin dynamics at the plasma membrane, during the assembly, maintenance and disassembly of the actin network (reviewed in Jockusch et al., 2007). Previous studies revealed that mice deficient in PFN1 fail to develop beyond the blastocyst stage (Witke et al., 2001) and gene silencing of PFN1 leads to a reduction of the actin filament formation and inhibits cell migration and the morphogenesis of endothelial cells (Ding et al., 2006). These profilin-dependent processes are due to its direct interaction with actin in the cellular environment, through regulating the actin dynamic with catalyzing the exchange of ADP for ATP and refilling the pool of ATP-actin monomers bound to profilin, ready for elongation (Pollard and Borisy, 2003). Furthermore, it was shown that changes in neuronal structure depends on changes in actin dynamic (Matus et al., 2000). However, despite their described role in regulating actin dynamics, the cell-specific role of both profiling isoforms remains largely elusive.

Recently, the *in vivo* role in synaptic physiology of PFN2a was addressed by using a PFN2a knockout mouse. The authors showed that while PFN2a is involved in regulating the presynaptic function of glutamatergic neurons by controlling vesicle exocytosis and presynaptic excitability, dendritic spine morphology and synaptic plasticity in these neurons are unaltered (Pilo-Boyl P. et al., 2007). In a second study, the conditional deletion of PFN1 revealed no alteration in neuronal morphology in the hippocampus (Gorlich et al., 2012). The mild or absent effects on neuronal architecture of PFN deletion, in both mice, could be explained by the compensatory up regulation of other Profilin isoform.

A previous study performed in our laboratory using an acute knockdown of PFN2a using an RNAi (shPFN2a) approach (Michaelsen, 2009), revealed a reduced dendritic complexity and reduced spine density for the mid apical and basal dendritic compartment of PFN2a depleted CA1 hippocampal neurons. These observations lead to two different possibilities. On the one hand, the formation of dendrites and spines could be inhibited or on the other hand already established dendrites and spines could retract upon PFN2a

knockdown. To this aim, time lapse imaging was used in this study to document the dendritic length and spine density of CA1 hippocampal neurons at different time points after PFN2a knockdown. In this experimental setting, both the basal and mid apical dendrites became progressively shorter or were completely retracted, suggesting that previously developed dendrites are losing their stability, retracting or even being pruned. Interestingly, the spine density of PFN2a-deficient neurons was not increasing during the imaged time period, as observed in control neurons, indicating that after PFN2a knockdown the spine formation is impaired. These data confirm the earlier analysis performed in neurons fixed 9 days after shPFN2a expression and showing dendritic complexity and spine density to be significantly decreased in the basal and mid apical dendritic compartment of CA1 hippocampal neurons (Michaelsen, 2009;Michaelsen et al., 2010). Moreover, the results of the time lapse imaging indicate a role for PFN2a in regulating the stability and maintenance of dendrites rather than their growth and in the formation of new spines rather than their maintenance. It is known that the PFN2a-mediated control of actin stability is regulated *via* a small GTPases RhoA and its downstream kinase ROCK (Da Silva et al., 2003). PFN2a can be phosphorylated *via* ROCK, but the consequences for the activation of PFN2a are still unclear (Da Silva et al., 2003;Sathish et al., 2004;Shao et al., 2008). However, the overexpression of a constitutively active RhoA results in neurite retraction in developing pyramidal neurons and decreased spine density (Nakayama et al., 2000). In relation to our results, this suggests that upon activation of RhoA the phosphorylation of profilin inhibits the profilin action in actin-mediated regulation of neuronal structure. However, to further investigate the mechanism how profilin might regulate dendrite stability a gene replacement approach with different phosphomutants could be used. In agreement with previous studies in non-neuronal cells where profilin is shown to stabilize the microfilament system (Rothkegel et al., 1996;Finkel et al., 1994), these data show that PFN2a is crucial for the stabilization of neuronal morphology most likely by stabilizing the actin cytoskeleton.

PFN2a is involved in spine stability

The major component of dendritic spines is F-actin (Matus et al., 1982). It was reported, that the dendritic spine formation (reviewed in Ethell and Pasquale, 2005) as well as their shape and stability (reviewed in Halpain, 2000) are dependent on stable as well as dynamic actin fibers. In this study, the spine type distribution of different dendritic

compartments was analyzed in order to address the question of whether, besides the impaired spine density also alterations of the spine size and shape could be observed in PFN2a-deficient neurons. After PFN2a knockdown, the spine type distribution was altered, too. Although the dendritic complexity and the spine density were impaired in both, the apical and basal dendritic compartments, the shift in spine types was different in the basal and apical dendrites of CA1 PFN2a-deficient cells. A possible explanation could be that PFN2a is not equally distributed throughout the dendritic tree of CA1 cells. In this case, one dendritic compartment of CA1 neurons could be affected by the depletion of PFN2a while the other compartment could compensate this effect with an opposite homeostatic regulation. Recently it was reported, that indeed a differential distribution of proteins, like MAP kinase or protein kinase M zeta could be observed between the apical and basal dendritic compartment (Sajikumar et al., 2007). As a differential effect of PFN2a knockdown between the apical and basal dendritic compartment of PFN2a-deficient neurons could be seen only for the spine type distribution and not for spine number, it is possible that the fine tuning of spines shape is more sensitive to alterations in the levels of PFN2a. However, to get more insight whether the different spine type distribution is due to different amounts of PFN2a in different CA1 neuronal compartments, the endogenous levels of PFN2a throughout the CA1 cell should be analyzed. The impaired spine shape after PFN2a knockdown may suggest an impaired spine dynamic. Recently, it could be shown that dendritic spines are continuously changing their size and shape and that their motility is based on the rearrangement of the actin cytoskeleton by actin polymerization and depolymerization (Fischer et al., 1998). However, the detailed mechanisms and key molecules regulating actin-dependent spine dynamics are not yet resolved. Therefore, the spine dynamics were determined in shPFN2a-expressing cells. Remarkably, I could observe that the spine motility after PFN2a knockdown was significantly increased in both dendritic compartments, suggesting a stabilizing role of PFN2a in spines. Is was reported, that based on their turnover rate two different F-actin filament pools could be identified throughout the dendritic spine under basal conditions (Honkura et al., 2008). The stable F-actin pool (turnover in 17 min) with more linear filaments is localized closer to the spine neck, while the dynamic F-actin pool is found in the spine head (turnover in 40 s) with branched F-actin filaments. Moreover a third F-actin pool, the enlargement pool (turnover in 2-15 min), could be observed in the spine head after incoming stimuli. However, the spine shape at rest is most likely maintained by keeping the balance between the stable

and the dynamic pool with different actin-binding proteins enhancing the stability or the dynamic of actin filaments (Bramham, 2008;Dominguez, 2009). So far, the role of PFN2a in maintaining a stable spine structure *via* regulating actin filaments remains unclear. Presumably, the increased spine mobility determined after PFN2a knockdown might be the result of an altered ratio between the stable and dynamic F-actin pool in the dendritic spine. Thus, an impairment in actin filament formation and stabilization due to the lack of PFN2a could result in a shrinkage of spines which in turn might lead to a subsequent homeostatic increase in spine size resulting in increased spine dynamics. This hypothesis could be investigated using FRAP experiment to determine different F-actin pools in spines after PFN2a knockdown. Interestingly, our preliminary data showed that the overexpression of an RNAi-resistant PFN2a in PFN2a-deficent neurons could partly rescue the spine head mobility, but was still significantly higher than in control neurons. This is consistent with previous studies where an overexpression of PFN2a stabilized dendritic spines (Ackermann and Matus, 2003). However, to further investigate why the RNAi-resistant PFN2a could rescue only in part the motility the spine motility after a plain overexpression of PFN2a should be analyzed. Possibly, the effect of impaired actin stabilization is similar upon PFN2a-knockdown and destabilization of the microfilament or after PFN2a-overexpression and hyperstabilization of the actin-filaments. Indeed, it could be shown that the same effect of impaired spine morphology is obtained after both, knockdown or after overexpression of MARCKS (myristoylated, alanine-rich C-kinase substrate), a protein proposed to be involved in regulating F-actin contents in spines via directly binding or crosslinking F-actin (Calabrese and Halpain, 2005). Thus, the actin dynamics seem to be a tightly regulated process and it is possible that both removing or adding an actin binding protein prevents this regulation. So far, the data in this study suggest that a proper level of PFN2a is important to stabilize resting dendritic spines in their mobility.

4.5. Profilin2a is involved in structural plasticity

In the next set of experiments the involvement of PFN2a in structural plasticity was investigated. Previous studies reported an activity-dependent targeting of PFN1 and PFN2a to dendritic spines (Ackermann and Matus, 2003;Neuhoff et al., 2005). However, the precise role of both profilins in structural plasticity remained so far unclear. It was reported, that upon high frequency stimulation a shift of the G-Actin/F-actin ratio towards

F-actin could be observed in spines (Fukazawa et al., 2003). Profilin, a promoter of actin filament assembly, might play a role in this response. In the current study the role of PFN2a in activity-dependent structural plasticity was investigated using a NMDA-dependent chemical long-term potentiation (cLTP) induced with Glycin. (Shahi et al., 1993). This stimulus protocol is comparable with the induction of LTP *via* the common theta burst protocol (Fortin et al., 2010) used to study the cellular basis of learning and memory. As expected (Fortin et al., 2010), the induction of LTP throughout the organotypic hippocampal slices revealed an enlargement of the spine head in control neurons. In contrast, in shPFN2a-expressing cells this increased spine head diameter could not be observed, suggesting an important role for PFN2a in activity-dependent structural plasticity. It was shown, that after LTP induction two different phases are involved (Engert and Bonhoeffer, 1999). During the first phase new spines are developed or the volume of existing spines increased through forming an enlargement F-actin pool (Honkura et al., 2008), while during the second phase the modulated spines are stabilized (Kasai et al., 2010). In this work, the spine head diameter was determined 60 min after the cLTP induction, in the stabilizing phase. The data obtained in this study so far do not allow to distinguish whether PFN2a is important for the enlargement phase or for the stabilizing phase of spines after cLTP induction. It is possible that the spine enlargement in PFN2a-deficient cells occurs transiently directly after the stimulation but it cannot be stabilized over the time. To investigate whether PFN2a modulates the enlargement or the stabilizing phase of dendritic spines in an activity-dependent manner, a more acute stimulation, like glutamate uncaging should be used in shPFN2a expressing cells and document starting already directly after the stimulation. Furthermore, it was reported that after KCl stimulation the PFN1 is as well targeted to dendritic spine head, (Neuhoff et al., 2005). It should be emphasized that in case of the KCl stimulation it is not clear if LTP is induced, as KCl induces a broad depolarization of neurons. In contrast, in the current work the Glycin stimulation is comparable to the common theta burst LTP induction (Fortin et al., 2010). Nevertheless, the results described here suggest that the endogenous PFN1 in PFN2a-deficent neurons is not sufficient for the spine head enlargement upon cLTP induction indicating a specific function of PFN2a. However, the amount of PFN1 might play a role in compensating the PFN2a action in activity-dependent structural plasticity. It could be shown that overexpressing PFN1 in PFN2a-deficeint neurons can rescue the reduced spine density (Michaelsen et al., 2010). An additional experiment with

cLTP induction in neurons expressing shPFN2a and overexpressing PFN1 would reveal further details.

Taken together, the results from this study, obtained combining an acute knockdown of PFN2a and time lapse imaging, provide evidence for a crucial role of PFN2a in maintaining the dendritic structure and in the formation of dendritic spines in CA1 hippocampal neurons. Moreover, the data indicate that PFN2a mediates the spine stability. Furthermore, new insights could be gained in describing a modulating role of PFN2a during activity-dependent structural plasticity.

5. Conclusion and Outlook

In the current study, I was able to show that the transactivation of the TrkB receptor in a neurotrophin-independent *via* Zinc has a biological relevance *in vitro* in regulating the neuronal morphology of excitatory neurons, but not the one of inhibitory neurons. The Zinc mediated regulation is different in dendrites and spines. BDNF and Zinc cooperate to regulate dendritic structure, while the spine density can be regulated by either one or the other. In contrast to spine density, the spine shape modulation seems to be done exclusively by BDNF. However, the observed results were obtained *in vitro*. To determine whether the same cell specific effect of BDNF could be observed *in vivo*, inhibitory neurons in different brain regions of *cbdnf ko* mice, especially in the hippocampus and cortex where excitatory neurons showed mild effects in their morphology *in vivo* and inhibitory neurons revealed a significantly reduced neuronal morphology *in vitro*, could be visualized by breeding GAD65-eGFP transgenic mice (Lopez-Bendito et al., 2004) with *cbdnf ko* mice. In GAD65-eGFP transgenic mice, the eGFP expression is driven by the GAD65 promoter, in most of the GABAergic neurons throughout the CNS (Fukuda et al., 1997). Next it is crucial to address the mechanism mediating the differences in the action of BDNF and Zinc in excitatory *versus* inhibitory neurons. One possible explanation for the complete dependence of inhibitory neurons from BDNF is that Zinc coming from excitatory neurons is released only on excitatory neurons and not on inhibitory neurons. Indeed, it could be shown that Zinc is released from excitatory neurons together with glutamate (Frederickson et al., 2005) at the synapses between the mossy fiber terminals and the CA3 pyramidal neurons in the hippocampus. A new method, the fluorescence resonance energy transfer (FRET) is reported to detect the mitochondrial Zinc (Sreenath et al., 2011). The FRET construct could be genetically modified to detect Zinc in the presynaptic terminals and distinguish whether Zinc is released on inhibitory neurons (labeled by eGFP expression under a specific promoter) as well. If Zinc is also released on inhibitory synapses another hypothesis for the different effect of Zinc on inhibitory and excitatory neurons, is that the transporter of Zinc (ZnT1-4) (reviewd in Kambe, 2011) might be different in excitatory and inhibitory neurons. To investigate this possibility, an immunhistochemical staining for different zinc transporters could be used. Finally, the biological function of the TrkB signaling activated by different molecules, BDNF and Zinc, but acting side by side on the same cell should be addressed in future studies. It is in fact possible that the downstream pathways might be different in the BDNF or Zinc induced TrkB signaling. BDNF-TrkB

signaling is indispensable for spine shape regulation. In this context it is possible that the TrkB phosphorylation upon BDNF binding could activate signaling pathways to regulate the actin cytoskeleton, as suggested previously (Murk et al., 2012). On the other hand, the transactivation of TrkB *via* Zinc could rather activate signaling pathways to regulate both the actin cytoskeleton and the microtubule stabilization, as Zinc is important for regulating dendritic structure and spine density. To investigate this hypothesis the microtubule dynamics after Zinc removal could be analyzed using time lapse imaging of a microtubules end-binding protein (EB3) tagged with GFP (Stepanova et al., 2003). If Zinc is involved in microtubule stabilization, the dynamics would increase and destabilize the dendritic structure after removal of Zinc.

In the second part of this study, I could show that the actin binding protein profilin2a (PFN2a) is important to regulate the neuronal morphology. PFN2a is crucial for maintaining the dendritic complexity, and for promoting the formation of new dendritic spines. Moreover, I identified PFN2a as a key modulator of activity-dependent structural plasticity. However, the mechanism by which PFN2a contributes to structural plasticity at spines is still unresolved. So far, I addressed the stabilizing phase in the activity-dependent structural plasticity. Whether PFN2a is important for the enlargement phase or for the stabilizing phase of spines after cLTP induction, a more acute stimulation like glutamate uncaging, could be used to distinguish between the two phase after stimulation. Moreover, PFN2a can be phosphorylated, but so far the consequences of this process are still unclear (Da Silva et al., 2003;Sathish et al., 2004;Shao et al., 2008). A gene replacement approach of PFN2a carrying a mutation in different phosphorylation sites of PFN2a could be used to determine the mechanism how PFN2a is involved in structural plasticity Furthermore, it was reported that the other brain specific isoforms, profilin1 (PFN1) is able to rescue the reduced spine density in PFN2a-deficient neurons (Michaelsen et al., 2010). To investigate whether PFN1 could compensate the impaired activity-dependent structural plasticity in PFN2a-deficient cell, the gene replacement approach could be used to overexpress PFN1 in PFN2a-deficient cells.

6. References

Ackermann M, Matus A (2003) Activity-induced targeting of profilin and stabilization of dendritic spine morphology. Nat Neurosci 6:1194-1200.

Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, Lindsay RM, Wiegand SJ (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature 389:856-860.

Arlotta P, Molyneaux BJ, Jabaudon D, Yoshida Y, Macklis JD (2008) Ctip2 controls the differentiation of medium spiny neurons and the establishment of the cellular architecture of the striatum. J Neurosci 28:622-632.

Atwal JK, Massie B, Miller FD, Kaplan DR (2000) The TrkB-Shc site signals neuronal survival and local axon growth via MEK and P13-kinase. Neuron 27:265-277.

Bannister AP (2005) Inter- and intra-laminar connections of pyramidal cells in the neocortex. Neurosci Res 53:95-103.

Baquet ZC, Gorski JA, Jones KR (2004) Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci 24:4250-4258.

Barde YA, Edgar D, Thoenen H (1982) Purification of a new neurotrophic factor from mammalian brain. EMBO J 1:549-553.

Berkemeier LR, Winslow JW, Kaplan DR, Nikolics K, Goeddel DV, Rosenthal A (1991) Neurotrophin-5: a novel neurotrophic factor that activates trk and trkB. Neuron 7:857-866.

Bibel M, Barde YA (2000) Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev 14:2919-2937.

Bramham CR (2008) Local protein synthesis, actin dynamics, and LTP consolidation. Curr Opin Neurobiol 18:524-531.

Calabrese B, Halpain S (2005) Essential role for the PKC target MARCKS in maintaining dendritic spine morphology. Neuron 48:77-90.

Carlsson L, Nystrom LE, Sundkvist I, Markey F, Lindberg U (1977) Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. J Mol Biol 115:465-483.

Carpenter G (1999) Employment of the epidermal growth factor receptor in growth factor-independent signaling pathways. J Cell Biol 146:697-702.

Castren E, Zafra F, Thoenen H, Lindholm D (1992) Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc Natl Acad Sci U S A 89:9444-9448.

Chan JP, Cordeira J, Calderon GA, Iyer LK, Rios M (2008) Depletion of central BDNF in mice impedes terminal differentiation of new granule neurons in the adult hippocampus. Mol Cell Neurosci 39:372-383.

Chan JP, Unger TJ, Byrnes J, Rios M (2006) Examination of behavioral deficits triggered by targeting Bdnf in fetal or postnatal brains of mice. Neuroscience 142:49-58.

Chao MV (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci 4:299-309.

Cingolani LA, Goda Y (2008) Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. Nat Rev Neurosci 9:344-356.

Cooper JA (1991) The role of actin polymerization in cell motility. Annu Rev Physiol 53:585-605.

Da Silva JS, Medina M, Zuliani C, Di NA, Witke W, Dotti CG (2003) RhoA/ROCK regulation of neuritogenesis via profilin IIa-mediated control of actin stability. J Cell Biol 162:1267-1279.

Dikic I, Batzer AG, Blaikie P, Obermeier A, Ullrich A, Schlessinger J, Margolis B (1995) Shc binding to nerve growth factor receptor is mediated by the phosphotyrosine interaction domain. J Biol Chem 270:15125-15129.

Ding Z, Lambrechts A, Parepally M, Roy P (2006) Silencing profilin-1 inhibits endothelial cell proliferation, migration and cord morphogenesis. J Cell Sci 119:4127-4137.

Dominguez R (2009) Actin filament nucleation and elongation factors--structure-function relationships. Crit Rev Biochem Mol Biol 44:351-366.

Dreznjak A (2008) Die Auswirkung des Neurotrophins BDNF auf die Morphologie von inhibitorischen und exzitatorischen Neuronen im Gehirn der Maus.

Engert F, Bonhoeffer T (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. Nature 399:66-70.

Ernfors P, Ibanez CF, Ebendal T, Olson L, Persson H (1990) Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: developmental and topographical expression in the brain. Proc Natl Acad Sci U S A 87:5454-5458.

Ethell IM, Pasquale EB (2005) Molecular mechanisms of dendritic spine development and remodeling. Prog Neurobiol 75:161-205.

Fan G, Egles C, Sun Y, Minichiello L, Renger JJ, Klein R, Liu G, Jaenisch R (2000) Knocking the NT4 gene into the BDNF locus rescues BDNF deficient mice and reveals distinct NT4 and BDNF activities. Nat Neurosci 3:350-357.

Finkel T, Theriot JA, Dise KR, Tomaselli GF, Goldschmidt-Clermont PJ (1994) Dynamic actin structures stabilized by profilin. Proc Natl Acad Sci U S A 91:1510-1514.

Fischer M, Kaech S, Knutti D, Matus A (1998) Rapid actin-based plasticity in dendritic spines. Neuron 20:847-854.

Fortin DA, Davare MA, Srivastava T, Brady JD, Nygaard S, Derkach VA, Soderling TR (2010) Long-term potentiation-dependent spine enlargement requires synaptic Ca2+-permeable AMPA receptors recruited by CaM-kinase I. J Neurosci 30:11565-11575.

Frederickson CJ, Koh JY, Bush AI (2005) The neurobiology of zinc in health and disease. Nat Rev Neurosci 6:449-462.

Frederickson CJ, Suh SW, Koh JY, Cha YK, Thompson RB, LaBuda CJ, Balaji RV, Cuajungco MP (2002) Depletion of intracellular zinc from neurons by use of an extracellular chelator in vivo and in vitro. J Histochem Cytochem 50:1659-1662.

Frederickson CJ, Suh SW, Silva D, Frederickson CJ, Thompson RB (2000) Importance of zinc in the central nervous system: the zinc-containing neuron. J Nutr 130:1471S-1483S.

Fukazawa Y, Saitoh Y, Ozawa F, Ohta Y, Mizuno K, Inokuchi K (2003) Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo. Neuron 38:447-460.

Fukuda T, Heizmann CW, Kosaka T (1997) Quantitative analysis of GAD65 and GAD67 immunoreactivities in somata of GABAergic neurons in the mouse hippocampus proper (CA1 and CA3 regions), with special reference to parvalbumin-containing neurons. Brain Res 764:237-243.

Gorlich A, Zimmermann AM, Schober D, Bottcher RT, Sassoe-Pognetto M, Friauf E, Witke W, Rust MB (2012) Preserved morphology and physiology of excitatory synapses in profilin1-deficient mice. PLoS One 7:e30068.

Gorski JA, Zeiler SR, Tamowski S, Jones KR (2003) Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. J Neurosci 23:6856-6865.

Grutzendler J, Tsai J, Gan WB (2003) Rapid labeling of neuronal populations by ballistic delivery of fluorescent dyes. Methods 30:79-85.

Gulyas AI, Megias M, Emri Z, Freund TF (1999) Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. J Neurosci 19:10082-10097.

Halpain S (2000) Actin and the agile spine: how and why do dendritic spines dance? Trends Neurosci 23:141-146.

Harris KM, Jensen FE, Tsao B (1992) Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. J Neurosci 12:2685-2705.

He XP, Butler L, Liu X, McNamara JO (2006) The tyrosine receptor kinase B ligand, neurotrophin-4, is not required for either epileptogenesis or tyrosine receptor kinase B activation in the kindling model. Neuroscience 141:515-520.

He XP, Kotloski R, Nef S, Luikart BW, Parada LF, McNamara JO (2004) Conditional deletion of TrkB but not BDNF prevents epileptogenesis in the kindling model. Neuron 43:31-42.

Hofer M, Pagliusi SR, Hohn A, Leibrock J, Barde YA (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 9:2459-2464.

Hohn A, Leibrock J, Bailey K, Barde YA (1990) Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. Nature 344:339-341.

Honkura N, Matsuzaki M, Noguchi J, Ellis-Davies GC, Kasai H (2008) The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. Neuron 57:719-729.

Horch HW, Kruttgen A, Portbury SD, Katz LC (1999) Destabilization of cortical dendrites and spines by BDNF. Neuron 23:353-364.

Hotulainen P, Hoogenraad CC (2010) Actin in dendritic spines: connecting dynamics to function. J Cell Biol 189:619-629.

Hu E, Chen Z, Fredrickson T, Zhu Y (2001) Molecular cloning and characterization of profilin-3: a novel cytoskeleton-associated gene expressed in rat kidney and testes. Exp Nephrol 9:265-274.

Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24:677-736.

Huang EJ, Reichardt LF (2003) Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 72:609-642.

Huang YZ, McNamara JO (2010) Mutual regulation of Src family kinases and the neurotrophin receptor TrkB. J Biol Chem 285:8207-8217.

Huang YZ, Pan E, Xiong ZQ, McNamara JO (2008) Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. Neuron 57:546-558.

Huber AB, Kolodkin AL, Ginty DD, Cloutier JF (2003) Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. Annu Rev Neurosci 26:509-563.

Ip NY, Li Y, Yancopoulos GD, Lindsay RM (1993) Cultured hippocampal neurons show responses to BDNF, NT-3, and NT-4, but not NGF. J Neurosci 13:3394-3405.

Ishizuka N, Weber J, Amaral DG (1990) Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. J Comp Neurol 295:580-623.

Ivkovic S, Ehrlich ME (1999) Expression of the striatal DARPP-32/ARPP-21 phenotype in GABAergic neurons requires neurotrophins in vivo and in vitro. J Neurosci 19:5409-5419.

Ji Y, Lu Y, Yang F, Shen W, Tang TT, Feng L, Duan S, Lu B (2010) Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. Nat Neurosci 13:302-309.

Ji Y, Pang PT, Feng L, Lu B (2005) Cyclic AMP controls BDNF-induced TrkB phosphorylation and dendritic spine formation in mature hippocampal neurons. Nat Neurosci 8:164-172.

Jockusch BM, Murk K, Rothkegel M (2007) The profile of profilins. Rev Physiol Biochem Pharmacol 159:131-149.

Jones TA, Klintsova AY, Kilman VL, Sirevaag AM, Greenough WT (1997) Induction of multiple synapses by experience in the visual cortex of adult rats. Neurobiol Learn Mem 68:13-20.

Kambe T (2011) An overview of a wide range of functions of ZnT and Zip zinc transporters in the secretory pathway. Biosci Biotechnol Biochem 75:1036-1043.

Kaplan DR, Miller FD (2000) Neurotrophin signal transduction in the nervous system. Curr Opin Neurobiol 10:381-391.

Kasai H, Fukuda M, Watanabe S, Hayashi-Takagi A, Noguchi J (2010) Structural dynamics of dendritic spines in memory and cognition. Trends Neurosci 33:121-129.

Katz LC, Gilbert CD, Wiesel TN (1989) Local circuits and ocular dominance columns in monkey striate cortex. J Neurosci 9:1389-1399.

Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133-1138.

Kellner Y (2010) Die Rolle des BDNF-TrkB Signalweges in synapitschen Strukturen hippocampaler Neurone.

Kohara K, Kitamura A, Adachi N, Nishida M, Itami C, Nakamura S, Tsumoto T (2003) Inhibitory but not excitatory cortical neurons require presynaptic brain-derived neurotrophic factor for dendritic development, as revealed by chimera cell culture. J Neurosci 23:6123-6131.

Kolbeck R, Bartke I, Eberle W, Barde YA (1999) Brain-derived neurotrophic factor levels in the nervous system of wild-type and neurotrophin gene mutant mice. J Neurochem 72:1930-1938.

Korets-Smith E, Lindemann L, Tucker KL, Jiang C, Kabacs N, Belteki G, Haigh J, Gertsenstein M, Nagy A (2004) Cre recombinase specificity defined by the tau locus. Genesis 40:131-138.

Korobova F, Svitkina T (2010) Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis. Mol Biol Cell 21:165-176.

Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T (1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci U S A 92:8856-8860.

Korte M, Minichiello L, Klein R, Bonhoeffer T (2000) Shc-binding site in the TrkB receptor is not required for hippocampal long-term potentiation. Neuropharmacology 39:717-724.

Krucker T, Siggins GR, Halpain S (2000) Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. Proc Natl Acad Sci U S A 97:6856-6861.

Lambrechts A, Braun A, Jonckheere V, Aszodi A, Lanier LM, Robbens J, Van C, I, Vandekerckhove J, Fassler R, Ampe C (2000) Profilin II is alternatively spliced, resulting in profilin isoforms that are differentially expressed and have distinct biochemical properties. Mol Cell Biol 20:8209-8219.

Lamprecht R, Farb CR, Rodrigues SM, LeDoux JE (2006) Fear conditioning drives profilin into amygdala dendritic spines. Nat Neurosci 9:481-483.

Lamprecht R, LeDoux J (2004) Structural plasticity and memory. Nat Rev Neurosci 5:45-54.

Lang SB, Stein V, Bonhoeffer T, Lohmann C (2007) Endogenous brain-derived neurotrophic factor triggers fast calcium transients at synapses in developing dendrites. J Neurosci 27:1097-1105.

Lee FS, Chao MV (2001) Activation of Trk neurotrophin receptors in the absence of neurotrophins. Proc Natl Acad Sci U S A 98:3555-3560.

Lee R, Kermani P, Teng KK, Hempstead BL (2001) Regulation of cell survival by secreted proneurotrophins. Science 294:1945-1948.

Levenson CW, Morris D (2011) Zinc and neurogenesis: making new neurons from development to adulthood. Adv Nutr 2:96-100.

Levi-Montalcini R (1987) The nerve growth factor: thirty-five years later. EMBO J 6:1145-1154.

Lewin GR, Barde YA (1996) Physiology of the neurotrophins. Annu Rev Neurosci 19:289-317.

Li XG, Somogyi P, Ylinen A, Buzsaki G (1994) The hippocampal CA3 network: an in vivo intracellular labeling study. J Comp Neurol 339:181-208.

Lopez-Bendito G, Sturgess K, Erdelyi F, Szabo G, Molnar Z, Paulsen O (2004) Preferential origin and layer destination of GAD65-GFP cortical interneurons. Cereb Cortex 14:1122-1133.

Lu B, Pang PT, Woo NH (2005) The yin and yang of neurotrophin action. Nat Rev Neurosci 6:603-614.

Maisonpierre PC, Belluscio L, Friedman B, Alderson RF, Wiegand SJ, Furth ME, Lindsay RM, Yancopoulos GD (1990) NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron 5:501-509.

Maisonpierre PC, Le Beau MM, Espinosa R, III, Ip NY, Belluscio L, de la Monte SM, Squinto S, Furth ME, Yancopoulos GD (1991) Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations. Genomics 10:558-568.

Marty S, Carroll P, Cellerino A, Castren E, Staiger V, Thoenen H, Lindholm D (1996) Brain-derived neurotrophic factor promotes the differentiation of various hippocampal nonpyramidal neurons, including Cajal-Retzius cells, in organotypic slice cultures. J Neurosci 16:675-687.

Marty S, Wehrle R, Sotelo C (2000) Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. J Neurosci 20:8087-8095.

Matsumoto T, Numakawa T, Yokomaku D, Adachi N, Yamagishi S, Numakawa Y, Kunugi H, Taguchi T (2006) Brain-derived neurotrophic factor-induced potentiation of glutamate and GABA release: different dependency on signaling pathways and neuronal activity. Mol Cell Neurosci 31:70-84.

Matsumoto T, Rauskolb S, Polack M, Klose J, Kolbeck R, Korte M, Barde YA (2008) Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat Neurosci 11:131-133.

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. Nature 429:761-766.

Matus A (2000) Actin-based plasticity in dendritic spines. Science 290:754-758.

Matus A, Ackermann M, Pehling G, Byers HR, Fujiwara K (1982) High actin concentrations in brain dendritic spines and postsynaptic densities. Proc Natl Acad Sci U S A 79:7590-7594.

Matus A, Brinkhaus H, Wagner U (2000) Actin dynamics in dendritic spines: a form of regulated plasticity at excitatory synapses. Hippocampus 10:555-560.

McAllister AK (2000) Cellular and molecular mechanisms of dendrite growth. Cereb Cortex 10:963-973.

McAllister AK, Katz LC, Lo DC (1996) Neurotrophin regulation of cortical dendritic growth requires activity. Neuron 17:1057-1064.

McAllister AK, Katz LC, Lo DC (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. Neuron 18:767-778.

McAllister AK, Katz LC, Lo DC (1999) Neurotrophins and synaptic plasticity. Annu Rev Neurosci 22:295-318.

McAllister AK, Lo DC, Katz LC (1995) Neurotrophins regulate dendritic growth in developing visual cortex. Neuron 15:791-803.

McBain CJ, Fisahn A (2001) Interneurons unbound. Nat Rev Neurosci 2:11-23.

Michaelsen K (2009) Molecular mechanisms regulating dendrite architecture of mature pyramidal neurons in the mouse hippocampus.

Michaelsen K, Murk K, Zagrebelsky M, Dreznjak A, Jockusch BM, Rothkegel M, Korte M (2010) Fine-tuning of neuronal architecture requires two profilin isoforms. Proc Natl Acad Sci U S A 107:15780-15785.

Minichiello L, Casagranda F, Tatche RS, Stucky CL, Postigo A, Lewin GR, Davies AM, Klein R (1998) Point mutation in trkB causes loss of NT4-dependent neurons without major effects on diverse BDNF responses. Neuron 21:335-345.

Mizuno K, Carnahan J, Nawa H (1994) Brain-derived neurotrophic factor promotes differentiation of striatal GABAergic neurons. Dev Biol 165:243-256.

Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, Parada LF, Nestler EJ (2007) Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biol Psychiatry 61:187-197.

Murk K, Wittenmayer N, Michaelsen-Preusse K, Dresbach T, Schoenenberger CA, Korte M, Jockusch BM, Rothkegel M (2012) Neuronal profilin isoforms are addressed by different signalling pathways. PLoS One 7:e34167.

Nagappan G, Woo NH, Lu B (2008) Ama "zinc" link between TrkB transactivation and synaptic plasticity. Neuron 57:477-479.

Nagappan G, Zaitsev E, Senatorov VV, Jr., Yang J, Hempstead BL, Lu B (2009) Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. Proc Natl Acad Sci U S A 106:1267-1272.

Nagerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T (2004) Bidirectional activity-dependent morphological plasticity in hippocampal neurons. Neuron 44:759-767.

Nakayama AY, Harms MB, Luo L (2000) Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. J Neurosci 20:5329-5338.

Neuhoff H, Sassoe-Pognetto M, Panzanelli P, Maas C, Witke W, Kneussel M (2005) The actin-binding protein profilin I is localized at synaptic sites in an activity-regulated manner. Eur J Neurosci 21:15-25.

Neuser SF (2010) Integration of ES cell-derived neurons into pre-existing neuronal circuits.

Ninkina N, Grashchuck M, Buchman VL, Davies AM (1997) TrkB variants with deletions in the leucine-rich motifs of the extracellular domain. J Biol Chem 272:13019-13025.

Obermann H, Raabe I, Balvers M, Brunswig B, Schulze W, Kirchhoff C (2005) Novel testis-expressed profilin IV associated with acrosome biogenesis and spermatid elongation. Mol Hum Reprod 11:53-64.

Okamoto K, Nagai T, Miyawaki A, Hayashi Y (2004) Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat Neurosci 7:1104-1112.

Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER (1996) Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron 16:1137-1145.

Penrod RD, Kourrich S, Kearney E, Thomas MJ, Lanier LM (2011) An embryonic culture system for the investigation of striatal medium spiny neuron dendritic spine development and plasticity. J Neurosci Methods 200:1-13.

Pilo-Boyl P., Di NA, Mulle C, Sassoe-Pognetto M, Panzanelli P, Mele A, Kneussel M, Costantini V, Perlas E, Massimi M, Vara H, Giustetto M, Witke W (2007) Profilin2 contributes to synaptic vesicle exocytosis, neuronal excitability, and novelty-seeking behavior. EMBO J 26:2991-3002.

Pollard TD, Borisy GG (2003) Cellular motility driven by assembly and disassembly of actin filaments. Cell 112:453-465.

Poo MM (2001) Neurotrophins as synaptic modulators. Nat Rev Neurosci 2:24-32.

Purves D, Snider WD, Voyvodic JT (1988) Trophic regulation of nerve cell morphology and innervation in the autonomic nervous system. Nature 336:123-128.

Rauskolb S (2008) Brain-derived neurotrophic factor: Generation and characterization of adult mice lacking BDNF in the adult brain.

Rauskolb S, Zagrebelsky M, Dreznjak A, Deogracias R, Matsumoto T, Wiese S, Erne B, Sendtner M, Schaeren-Wiemers N, Korte M, Barde YA (2010) Global deprivation of brain-derived neurotrophic factor in the CNS reveals an area-specific requirement for dendritic growth. J Neurosci 30:1739-1749.

Reichardt LF (2006) Neurotrophin-regulated signalling pathways. Philos Trans R Soc Lond B Biol Sci 361:1545-1564.

Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, Lechan RM, Jaenisch R (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol Endocrinol 15:1748-1757.

Rothkegel M, Mayboroda O, Rohde M, Wucherpfennig C, Valenta R, Jockusch BM (1996) Plant and animal profilins are functionally equivalent and stabilize microfilaments in living animal cells. J Cell Sci 109 (Pt 1):83-90.

Rutherford LC, Nelson SB, Turrigiano GG (1998) BDNF has opposite effects on the quantal amplitude of pyramidal neuron and interneuron excitatory synapses. Neuron 21:521-530.

Sajikumar S, Navakkode S, Frey JU (2007) Identification of compartment- and process-specific molecules required for "synaptic tagging" during long-term potentiation and long-term depression in hippocampal CA1. J Neurosci 27:5068-5080.

Sathish K, Padma B, Munugalavadla V, Bhargavi V, Radhika KV, Wasia R, Sairam M, Singh SS (2004) Phosphorylation of profilin regulates its interaction with actin and poly (L-proline). Cell Signal 16:589-596.

Schluter K, Jockusch BM, Rothkegel M (1997) Profilins as regulators of actin dynamics. Biochim Biophys Acta 1359:97-109.

Schubert V, Da Silva JS, Dotti CG (2006) Localized recruitment and activation of RhoA underlies dendritic spine morphology in a glutamate receptor-dependent manner. J Cell Biol 172:453-467.

Seidah NG, Benjannet S, Pareek S, Chretien M, Murphy RA (1996a) Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. FEBS Lett 379:247-250.

Seidah NG, Benjannet S, Pareek S, Savaria D, Hamelin J, Goulet B, Laliberte J, Lazure C, Chretien M, Murphy RA (1996b) Cellular processing of the nerve growth factor precursor by the mammalian pro-protein convertases. Biochem J 314 (Pt 3):951-960.

Seil FJ, Drake-Baumann R (2000) TrkB receptor ligands promote activity-dependent inhibitory synaptogenesis. J Neurosci 20:5367-5373.

Shahi K, Marvizon JC, Baudry M (1993) High concentrations of glycine induce long-lasting changes in synaptic efficacy in rat hippocampal slices. Neurosci Lett 149:185-188.

Shao J, Welch WJ, Diprospero NA, Diamond MI (2008) Phosphorylation of profilin by ROCK1 regulates polyglutamine aggregation. Mol Cell Biol 28:5196-5208.

Shelton DL, Sutherland J, Gripp J, Camerato T, Armanini MP, Phillips HS, Carroll K, Spencer SD, Levinson AD (1995) Human trks: molecular cloning, tissue distribution, and expression of extracellular domain immunoadhesins. J Neurosci 15:477-491.

SHOLL DA (1953) Dendritic organization in the neurons of the visual and motor cortices of the cat. J Anat 87:387-406.

Sorensen SA, Rubel EW (2006) The level and integrity of synaptic input regulates dendrite structure. J Neurosci 26:1539-1550.

Spacek J, Harris KM (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. J Neurosci 17:190-203.

Spruston N (2008) Pyramidal neurons: dendritic structure and synaptic integration. Nat Rev Neurosci 9:206-221.

Sreenath K, Allen JR, Davidson MW, Zhu L (2011) A FRET-based indicator for imaging mitochondrial zinc ions. Chem Commun (Camb) 47:11730-11732.

Star EN, Kwiatkowski DJ, Murthy VN (2002) Rapid turnover of actin in dendritic spines and its regulation by activity. Nat Neurosci 5:239-246.

Stepanova T, Slemmer J, Hoogenraad CC, Lansbergen G, Dortland B, De Zeeuw CI, Grosveld F, van CG, Akhmanova A, Galjart N (2003) Visualization of microtubule growth in cultured neurons via the use of EB3-GFP (end-binding protein 3-green fluorescent protein). J Neurosci 23:2655-2664.

Stephens RM, Loeb DM, Copeland TD, Pawson T, Greene LA, Kaplan DR (1994) Trk receptors use redundant signal transduction pathways involving SHC and PLC-gamma 1 to mediate NGF responses. Neuron 12:691-705.

Stoppini L, Buchs PA, Muller D (1991) A simple method for organotypic cultures of nervous tissue. J Neurosci Methods 37:173-182.

Tanaka J, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GC, Kasai H (2008) Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. Science 319:1683-1687.

Tepper JM, Bolam JP (2004) Functional diversity and specificity of neostriatal interneurons. Curr Opin Neurobiol 14:685-692.

Thieme N (2011) Die Rolle des BDNF-TrkB Signalweges auf die dendritische Architektur von hippocampalen Neuronen.

Thoenen H (1995) Neurotrophins and neuronal plasticity. Science 270:593-598.

Timmusk T, Belluardo N, Metsis M, Persson H (1993) Widespread and developmentally regulated expression of neurotrophin-4 mRNA in rat brain and peripheral tissues. Eur J Neurosci 5:605-613.

Tucker KL, Meyer M, Barde YA (2001) Neurotrophins are required for nerve growth during development. Nat Neurosci 4:29-37.

Tyler WJ, Pozzo-Miller L (2003) Miniature synaptic transmission and BDNF modulate dendritic spine growth and form in rat CA1 neurones. J Physiol 553:497-509.

Tyler WJ, Pozzo-Miller LD (2001) BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses. J Neurosci 21:4249-4258.

Unger TJ, Calderon GA, Bradley LC, Sena-Esteves M, Rios M (2007) Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. J Neurosci 27:14265-14274.

Vetter ML, Martin-Zanca D, Parada LF, Bishop JM, Kaplan DR (1991) Nerve growth factor rapidly stimulates tyrosine phosphorylation of phospholipase C-gamma 1 by a kinase activity associated with the product of the trk protooncogene. Proc Natl Acad Sci U S A 88:5650-5654.

Vicario-Abejon C, Collin C, McKay RD, Segal M (1998) Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. J Neurosci 18:7256-7271.

Volkmar FR, Greenough WT (1972) Rearing complexity affects branching of dendrites in the visual cortex of the rat. Science 176:1445-1447.

von Bohlen und HO (2009) Structure and function of dendritic spines within the hippocampus. Ann Anat 191:518-531.

Wiese S, Jablonka S, Holtmann B, Orel N, Rajagopal R, Chao MV, Sendtner M (2007) Adenosine receptor A2A-R contributes to motoneuron survival by transactivating the tyrosine kinase receptor TrkB. Proc Natl Acad Sci U S A 104:17210-17215.

Witke W, Podtelejnikov AV, Di NA, Sutherland JD, Gurniak CB, Dotti C, Mann M (1998) In mouse brain profilin I and profilin II associate with regulators of the endocytic pathway and actin assembly. EMBO J 17:967-976.

Witke W, Sutherland JD, Sharpe A, Arai M, Kwiatkowski DJ (2001) Profilin I is essential for cell survival and cell division in early mouse development. Proc Natl Acad Sci U S A 98:3832-3836.

Yamada MK, Nakanishi K, Ohba S, Nakamura T, Ikegaya Y, Nishiyama N, Matsuki N (2002) Brain-derived neurotrophic factor promotes the maturation of GABAergic mechanisms in cultured hippocampal neurons. J Neurosci 22:7580-7585.

Yang J, Siao CJ, Nagappan G, Marinic T, Jing D, McGrath K, Chen ZY, Mark W, Tessarollo L, Lee FS, Lu B, Hempstead BL (2009) Neuronal release of proBDNF. Nat Neurosci 12:113-115.

Yuste R, Bonhoeffer T (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci 24:1071-1089.

Zafra F, Hengerer B, Leibrock J, Thoenen H, Lindholm D (1990) Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. EMBO J 9:3545-3550.

Zagrebelsky M, Holz A, Dechant G, Barde YA, Bonhoeffer T, Korte M (2005) The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons. J Neurosci 25:9989-9999.

Zhou Q, Homma KJ, Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron 44:749-757.

7. Table of figures

8. Abbreviations

BDNF	brain derived neurotrophic factor
CA1,3	cornu ammonis (hippocampal subfields)1,3
CaEDTA	calcium ethylenediaminetetraacetic acid
cbdnf ko	conditional *bdnf* knockout
ceGFP	cytoplasmatic enhanced green fluorescent protein
cLTP	chemical long-term potentiation
CNS	central nervous system
DIV	days in vitro
dpt	days post-transfection
fCherry	farnesylated red fluorescent protein mcherry
feGFP	farnesylated enhanced green fluorescent protein
GABA	γ-aminobutyric acid
LTD	long-term depression
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
MSNs	medium spiny neurons
NMDA	N-methy-D-aspartic acid
NT4	neurotophin 4
PFN1	profilin1
PFN2a	profilin2a
PLC γ	phospholipase C-γ
RNAi	RNA interference
shPFN2a	short hairpin RNA targeting profilin2a mRNA
TrkB	tropomyosin related kinase receptor B
TrkB Fc	tropomyosin related kinase receptor B Chimera
wt	wild type

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